Vol. 8 No. 4

PLANT PHYSIOLOGY

OCTOBER, 1933

CONCENTRATIONS OF INORGANIC IONS AS RELATED TO GROWTH OF EXCISED ROOT-TIPS OF WHEAT SEEDLINGS

PHILIP R. WHITE

(WITH SEVEN FIGURES)

Introduction

Growth of excised wheat root-tips, as influenced by H-ion concentration of the medium, volume of the medium, temperature, illumination, and organic nutrition was considered in an earlier paper (**10**). The present contribution deals with additional preliminary experiments on growth of the same kind of root-tips as related to concentrations and proportions of inorganic ions (other than H^+ and OH^-) in the medium.

Except as otherwise stated, experimental procedures were like those described in the paper just referred to: the culture flasks (each with a single root-tip) stood in a well ventilated basement room, with temperature maintained at about 25° C. and with continuous artificial illumination from nitrogen-filled Mazda "daylight" lamps; the standard nutrient medium used was the USPENSKI solution (**9**), to each liter of which 20 gm. of

TABLE I

CONCENTRATION OF SALTS USED

SALT	CONCENTRATION		
	MOLAR	NORMAL	MG. PER LITER
$Ca(NO_3)_2$	0.0006	0.0003	100
$MgSO_4$	0.0002	0.0001	25
K_2CO_3	0.00025	0.000125	35
KNO_3	0.00025	0.00025	25
KH_2PO_4	0.000185	0.000061	25
$Fe_2(SO_4)_3$	0.000003	0.000001	1.25

dextrose was added, with the extract of 0.1 gm. of dry brewers' yeast. The salts of the USPENSKI solution, together with their molar and normal concentrations and their concentrations in milligrams per liter, are shown in table I.

The total salt concentration was consequently about 0.0015 M., or 211.25 mg. per liter. Ionization is supposed to have been practically complete. Neglecting such small amounts of incompletely ionized salts as may have been present, as well as traces of unknown impurities in the chemicals used and unknown constituents of the yeast extract added, the standard medium may be taken to have contained, besides H^+ and OH^-, the following potential ions in approximately the partial concentrations shown.

TABLE II*

CONCENTRATION OF IONS IN SOLUTION

		CONCENTRATION PER LITER (MILLIGRAMS)		
		MG.-ION	MG. EQUIVALENT	MG.
Cations	K	0.940 (U_K)	0.940	36.70
	Ca	0.600 (U_{Ca})	0.300	24.00
	Mg	0.200 (U_{Mg})	0.100	5.00
	Fe	0.006 (U_{Fe})	0.002	0.35
Anions	NO_3	1.45 (U_{NO_3})	1.450	91.0
	SO_4	0.21 (U_{SO_4})	0.100	20.0
	CO_3	0.25 (U_{CO_3})	0.125	15.0
	PO_4	0.18 (U_{PO_4})	0.090	17.5

* In table II and throughout this paper, the symbols for the several kinds of ions appear without their plus or minus signs; thus K is used instead of K+, Fe is used instead of Fe^{+++}, SO_4 instead of SO_4^-, etc. Such usage avoids considerable expense in printing and cannot lead to ambiguity in such discussions as this.

In table II, each partial concentration is shown by three different values, each of which might be computed from either of the others: (1) "Milligram-ion per liter" is the quotient obtained by dividing the number of milligrams per liter, of the ion in question, by the ionic weight of the latter; it corresponds to the familiar "gram-molecules per liter." (2) "Milligram-equivalent per liter" is the quotient obtained by dividing "milligram-ion per liter" by the valence of the ion in question; it corresponds to the familiar "normal concentration." (3) "Milligrams per liter" is simply the weight, in milligrams, of the ion in question contained in a liter of medium; its value is identical with that of the corresponding concentration expressed as parts per million, being the corresponding percentage concentration multiplied by 10,000. Relative ionic concentrations rather than

relative molar concentrations or relative weights of elements, etc., per liter, are of primary interest in comparing nutrient media with respect to their physiological influence, as was emphasized in SHIVE'S discussion of ionic ratios (7); therefore the concentration values considered in this paper are always ionic. They are generally referred to the mg.-ion values just given, which serve as concentration units. Convenient designations for these units are shown in parentheses in table II; for example, 0.94 mg.-ion of K per liter (the standard partial concentration of K) is called U_K, 1.45 mg.-ion of $_{NO_3}$ per liter is called U_{NO_3}, etc. Consequently 0.5 U_{NO_3} represents 0.725 mg.-ion of NO_3 per liter, etc.

With the standard medium as a basis, various modifications were tested, these being made up with different concentrations of the ions considered. There were 127 of these modified media, giving a great number of differences in ion proportions. The influences exerted on growth by some of the main ions of the standard solution were thus studied separately or in pairs, as will be seen from the account of results. Each ion studied was employed at higher and lower concentrations than were specified for the standard medium, which was always used as control.

The complete omission of an ion in the preparation of a medium did not, of course, imply that the ion in question was entirely absent, for very small traces of all the main ions may be supposed to have been derived from salt impurities or from the yeast extract used. Media designated as "without K," for instance, probably had unknown but very small concentrations of K.

To study the ion relations of an organism it would naturally be desirable, among other things, to alter the concentration of each ion separately, without at the same time introducing any other alteration in the constitution of the nutrient medium; but this is obviously impossible, since ions cannot be handled separately, as is possible with molecules. In order to alter the concentration of any ion it is necessary to alter the concentration of one or more of the other ions also,—an obvious consideration which complicates matters exceedingly, although it has seldom been taken seriously into account in solution-culture experimentation (see MORITA and LIVINGSTON'S (5) plan for studying solution cultures of wheat plants in solutions from which potassium was omitted). In the present experiments on excised wheat root-tips, the concentration of any ion was decreased by suitably decreasing the concentration of its salt or salts and by adding a suitable amount, either of a sodium salt with the corresponding anion or of a chloride with the corresponding cation; and the concentration of any ion was increased simply by adding to the solution the requisite amount either of the corresponding sodium salt or of the corresponding chloride. By this procedure each modified medium was approximately like the standard medium with respect to the concentrations of all the ions of the latter, excepting the ion or ions

whose partial concentrations were purposely altered; but some of the modified solutions differed from the standard solution also with respect to their concentration of Na or of Cl, for these extra ions are not included in the USPENSKI solution. For the purposes of this preliminary study, it may be tentatively supposed, without prejudice, that these small additions of Na or of Cl were not significantly influential upon the growth of the root-tips.

Each culture flask, with 50 ml. of medium, had a single root-tip. The root-tips were measured after a period of 14 days, the length of the main root and the total length of all branches being recorded for each culture.

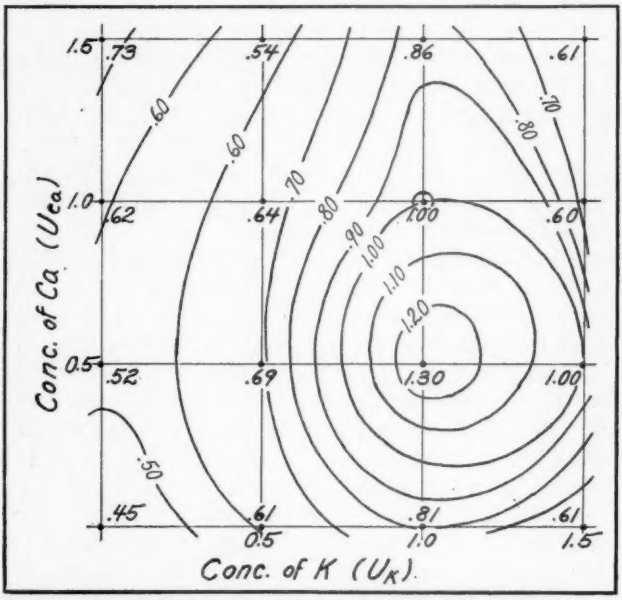

FIG. 1. Three-dimensional diagram showing by means of isopleths growth responses as related to different concentrations of K and Ca. Relative growth indices are inscribed at the points representing the several combinations of these ions.

The sum of these measurements gave the total increment of elongation for the culture in question. (Because the original excised tips, with which all cultures were started, were uniformly only about 3 mm. long, this original length may be considered as insignificant.) The growth increments for all like cultures in each series were averaged, to give a single numerical growth index for the particular solution employed. The relative physiological suitability of any medium tested may be judged by comparing its growth index with the corresponding growth index for the standard medium. Photo-

graphs of representative root-tips were made in many instances and records were kept as to general appearance and amount of branching.

Experimental results

Cations

The influence of K and Ca upon growth was studied in two similar series of cultures, carried out at about 26° C. Besides the standard medium there were 15 modifications, and each series comprised 10 cultures of each medium. The modified media were like the standard medium in ionic concentrations, excepting with respect to the concentrations of K and Ca (and, in

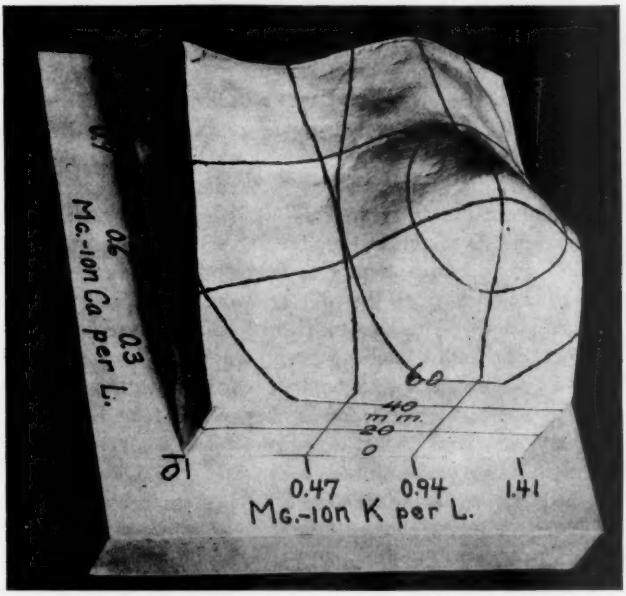

FIG. 2. Model showing the relations in figure 1, but concentrations are shown here as mg.-ions per liter and altitudes are actual growth indices, in mm.

some instances, with respect to added Na or Cl). The replacements were: K_2CO_3 by Na_2CO_3, KNO_3 by $NaNO_3$, KH_2PO_4 by NaH_2PO_4, and $Ca(NO_3)_2$ by $NaNO_3$. KNO_3 was supplemented by KCl, and $Ca(NO_3)_2$ was supplemented by $CaCl_2$. The concentrations of K and Ca in these series are shown on the diagram of figure 1. One medium received neither K nor Ca, three received no K, and three received no Ca. Those receiving K had K-ion concentration of 0.5 U_K, 1.0 U_K, or 1.5 U_K, while those receiving Ca had Ca-ion concentration of 0.5 U_{Ca}, 1.0 U_{Ca}, or 1.5 U_{Ca}.

The first of these two series was carried out in continuous darkness. The pH values of the media at the end of the culture period were all between 5.4 and 5.7, a range previously shown (**10**) to give excellent growth of these root-tips. The growth indices obtained, expressed in terms of the index for the standard medium (76.0 mm. = 1.00), are inscribed at the points on the diagram just mentioned. Each isopleth thus represents a series of K–Ca ratios that were about equally effective for growth of these root-tips. The solid diagram of figure 2 represents these results in another way.

Best growth occurred in the medium having a K concentration of U_K (*i.e.*, 0.94 mg-ion per liter) and a Ca concentration of 0.5 U_{Ca} (*i.e.*, 0.30 mg.-ion per liter). It thus appears that the standard medium might have given greater growth had its concentration of Ca been somewhat lower, but that its concentration of K was about optimal for the range of salt proportions represented by this experiment.

It is obvious that if a set of vertical or horizontal profiles were erected upon the diagram of figure 1, the growth relations shown by any profile might be very different from those shown by another profile of the same set. Any such profile is, of course, the concentration-growth curve as usually constructed when an ion is treated as an independent variable, and the failure of these curves to coincide under experimental complexes that differed only slightly and with respect to only one other variable emphasizes the necessity of considering all the effective variables in such studies as this.

In the second of these two series with K and Ca, carried out in diffuse natural daylight, the eight media represented by the points on the lower half of figure 1 gave results that agreed essentially with those obtained from the first series. The best growth, for the same medium as before, showed an absolute index value about 15 per cent. lower than in the first series. The final pH values of these media were between 5.2 and 5.6, about as in the first series. On the other hand, the eight media represented by the points on the upper half of the figure were found (for some unknown reason, perhaps connected with autoclaving) to have final pH values as high as 7.2, being thus much more alkaline than the corresponding media of the first series. The growth indices for these more alkaline media were much lower than those for media similar in constitution but with more acid reaction, as shown on the figure,—an observation which is in accord with an earlier conclusion (**10**) that these root-tips gave only relatively low growth indices when the pH value of the medium was higher than about 6.0. This unexplained difference in final pH values between two sets of media intended to be alike seems to emphasize the need for extreme care in the preparation and autoclaving of culture media for studies of this kind. These media were apparently very labile under some of the conditions to which they were subjected.

The partial concentration of Mg was varied in six series of cultures. Two of these were carried out in darkness, one in continuous weak artificial light, and three in more intense continuous artificial light such as was employed in earlier experiments on light influence (**10**). The first three had Mg concentrations of 0.0, 0.5, 1.0, and 1.5 U_{Mg}, and the last had concentrations of 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 U_{Mg}. The partial concentrations of the other ions were always the same as those specified for the standard medium (excepting Na and Cl in some cases). The medium without Mg contained Na_2SO_4 instead of $MgSO_4$; the media with Mg concentrations lower than 1 U_{Mg} contained both $MgSO_4$ and Na_2SO_4; and those with Mg concentrations higher than 1 U_{Mg} received additional Mg as $MgCl_2$.

The average growth index for all tests in which Mg was omitted was 45 per cent. greater than the corresponding average for the standard medium. When Mg was present at a concentration of 0.5 U_{Mg}, the average index was somewhat (8 per cent.) smaller than the corresponding control average, but this may not be a significant difference. With Mg concentration of 1.5 or 2.0 U_{Mg}, the respective averages for all series were 12 or 13 per cent. greater than the control average. With Mg concentration of 2.5 U_{Mg}, the average for all series was sensibly like the control average. Of course there was some deviation among the several series, but the detailed data generally indicate more growth in the medium without Mg than in any of the others. The relative growth indices for the medium without Mg in these experiments are as follows, the corresponding control index being taken as 1.00: For two series in continuous darkness, 0.89 and 1.18; for one series in weak continuous artificial light, 1.08; for three series in more intense continuous artificial light, 2.45, 1.48, and 1.63. A representative of the root-tips grown without Mg is shown at the left in figure 3; these were straight and of unusually great length, but generally only sparsely branched. A comparable representative root-tip grown in standard medium with Mg concentration of 1 U_{Mg}) is shown at the center of the figure; these control root-tips were much shorter and more crooked but had more branches than those grown without Mg. The right-hand root-tip in the figure represents those grown with an Mg concentration of 2 U_{Mg}; their growth habit was much like that of those grown without Mg, but their growth index is 49 per cent. less. All three of the root-tips were grown with temperature about 26° and with relatively intense continuous artificial illumination, the only difference in treatment being with respect to Mg concentration.

The medium without Mg was somewhat opalescent when taken from the autoclave, but precipitation soon ensued, leaving the liquid clear and colorless. On the other hand, all solutions with Mg were clear when taken from the autoclave, and they showed no subsequent precipitation but were somewhat yellowish in color, suggesting that some caramelization of dextrose may

FIG. 3. Representative root-tips grown 2 weeks in media differing only with respect to the concentration of Mg: *A*, without Mg; *B* and *C*, with 0.2 mg.-ion of Mg per liter (standard) and 0.4 mg.-ion of Mg per liter respectively. About natural size.

have occurred in the process of autoclaving. This difference between the medium without Mg and those with Mg was not paralleled by any significant difference in pH value. The yellow tint of the media with Mg was found to be progressively more pronounced as the concentration of K_2CO_3 was higher. These observations led to the suggestion that a medium with a concentration of CO_3 less than U_{CO_3} might perhaps give more vigorous growth than was given by the standard medium.

The concentrations of both Mg and CO_3 were therefore varied, in a single experiment which was carried out at about 24° and with continuous intense artificial illumination. Besides the standard medium there were in this series 17 modified media, and each of the 18 was represented by 10 cultures. The concentrations of all component ions excepting Mg and CO_3 (and in some cases Na and Cl) were the same for all these media. The 18 different combinations of Mg concentrations and CO_3 concentrations tested are shown in figure 4, where it is seen that Mg had concentrations of 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 U_{Mg}, and that CO_3 had concentrations of 0.0, 1.0, and 2.0 U_{CO_3}. The media from which CO_3 was omitted received KCl instead of the carbonate; those with CO_3 at concentrations lower than 1 U_{CO_3} received both K_2CO_3 and KCl; and those with CO_3 concentrations higher than 1 U_{CO_3} received both K_2CO_3 and Na_2CO_3.

The relative values of the growth indices obtained from this series are inscribed on the 3-dimensional diagram of figure 4, in which each contour line or isopleth indicates concentration combinations (Mg-CO_3 ratios) that gave like growth values. The hachured area represents media closely approaching the standard medium in effectiveness. The actual average index for the standard medium was 72.0 mm., which is taken as unity.

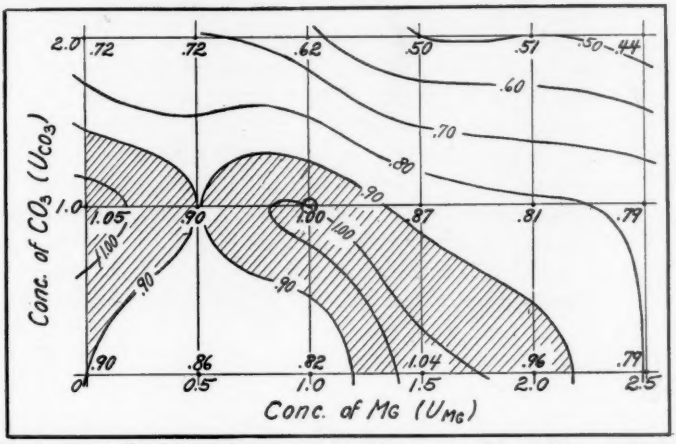

FIG. 4. Three-dimensional diagram showing by means of isopleths growth responses as related to different concentrations of Mg and CO_3; relative growth indices are inscribed at the points representing the several combinations of these ions. Hachured area represents media closely approaching the standard medium (1.00) in effectiveness.

With the exception of the single point whose coordinates are 0.5 U_{Mg}, 1 U_{CO_3}, the relations shown seem to be satisfactorily consistent; perhaps the growth index for the medium represented by that point may be erratically low. At any rate it appears that the standard medium was slightly surpassed, for growth in these 2-week tests, by two modified media, one without Mg (and with CO_3 concentration of 1 U_{CO_3}) and the other without CO_3 (and with Mg concentration of 1.5 U_{Mg}). The standard medium might apparently be simplified and somewhat improved by omitting Mg or by omitting CO_3 and at the same time increasing the Mg concentration by 50 per cent.

These suggestions may be added to the suggestion that the standard medium might be improved by decreasing its Ca concentration by 50 per cent. But it is to be remembered that the physiological influences due to the several ions are not to be regarded as independent variables; consequently it need not be supposed that the standard medium would be improved by simultaneously decreasing its Ca concentration and omitting Mg, or by

simultaneously decreasing its Ca concentration, increasing its Mg concentration, and omitting CO_3. A more elaborate series of tests than has thus far been attempted would be required to throw light on the question thus raised. In this connection it is to be borne in mind that such modifications of the standard medium would each necessarily involve still other resultant modifications with respect to the concentrations of ions other than those primarily considered.

Three different sources of iron were compared in five series of cultures with temperature of 22°–27°. The substances used were $Fe_2(SO_4)_3$ (the iron salt specified in the USPENSKI formula), Fe_2Cl_6, and a commercial preparation of uncertain composition called "iron citrate."[1] Ferric sulphate is not deliquescent, and specified concentrations of it may be prepared by simple weighing without special precautions; but it tends to form ferric hydroxide in solution, which is only slightly soluble. Ferric chloride, which has been used as iron source by many students of plant nutrition, is markedly deliquescent and it is usually undesirable to depend on weighed quantities of it for specified concentrations. Of course, measured quantities of a solution whose Fe content has been ascertained by analysis may be used in preparing nutrient media. In complex nutrient solutions, such as those of this study, each of these sources of iron may give concentrations of Fe that are markedly different from values computed on the basis of weighing and the formula, especially when the solutions are not decidedly acid. Despite these and similar troublesome considerations, the solutions used in these experiments were prepared in the ordinary way, by weighing, on the supposition that the three formulas were $Fe_2(SO_4)_3$, $Fe_2Cl_6 \cdot 12H_2O$, and $FeC_6H_5O_7 \cdot 3H_2O$. Influences on growth that might possibly be exerted by the very low concentrations of the anions of these salts were left out of account. All the constituents of these solutions, except the iron source, were at the respective concentrations specified for the standard medium. The Fe concentrations employed were 0.0, 0.5, 1.0, and 1.5 U_{Fe} (U_{Fe} being 0.006 mg.-ion per liter).

[1] The U. S. Pharmacopoeia, 7th ed. (6), gives "iron citrate" as $FeC_6H_5O_7 \cdot 3H_2O$ [?], after the formula of SCHIFF, while BEILSTEIN (1) gives forms with 1, 1½, and 3 molecules of water of crystallization as well as more complex formulas. BELLONI (2), who gave special attention to this material, stated that it is probably a dicitric-triferric citrate of the base $[Fe_3(C_6H_5O_7)_2(OH)_2]OH$, with the formula $[Fe_3(C_6H_5O_7)_2(OH)_2(OH_2)_2]\frac{1}{2}C_6H_5O_7 \cdot 6H_2O$. Among physiologists who have employed iron in citrate form, HOPKINS (3) used $KC_6H_5O_7$ and $FeCl_3$ together in his nutrient solution. He gives the formula of the resulting potassium-iron compound as $KH_3[(C_2H_4O_3)(CO_2)_3Fe_2]$, and states that he is "of the opinion that any preparation of ferric citrate purchased on the market would be of rather uncertain composition" (3a). A letter from the Chemical Division of the Eastman Kodak Co. refers to ferric ammonium citrate (which is used in photography) as $4FeC_6H_5O_7 \cdot 3(NH_4)_3C_6H_5O_7 \cdot 3Fe(OH)_3$.

Three series, one for each iron source, were carried out simultaneously, each series having 10 cultures with each Fe concentration. The cultures received only diffuse natural daylight. Relative values of the resulting growth indices are shown below. The actual average index for the standard medium (marked with an asterisk) was 64.4 mm., which is taken as unity. The medium without Fe gave an actual average index of 48.8 mm., or a relative index of 0.74, which is lower than any of the nine index values for solutions containing Fe.

Fe supplied as	Fe concentration (mg.-ion per liter)		
	0.003	0.006 (U_{Fe})	0.009
$Fe_2(SO_4)_3$	0.80	1.00*	1.48
Fe_2Cl_6	0.92	0.91	0.59
"Ferric citrate"	0.89	1.11	0.94

Only with the sulphate were the growth values regularly larger as the Fe concentration was higher. With Fe concentration of 0.003 mg.-ion per liter the chloride and the "citrate" were apparently both slightly superior to the sulphate. With Fe concentration of 0.006 mg.-ion per liter (U_{Fe}), the citrate was apparently slightly, although probably not significantly, superior to the sulphate and the chloride was apparently somewhat inferior to the sulphate. With Fe concentration of 0.009 mg.-ion per liter, both chloride and citrate were very much inferior to the sulphate. The indications are that these three sources of iron were about equally efficient when the Fe concentration was 0.5 U_{Fe} or 1 U_{Fe}, but that the citrate was unsatisfactory and the chloride was markedly toxic when the Fe concentration was 1.5 U_{Fe}.

The series with $Fe_2(SO_4)_3$ was repeated, with results almost exactly like those just stated for cultures in media containing iron in this form. Another like series with $Fe_2(SO_4)_3$, carried out in continuous darkness, gave an average growth index of 87.8 mm. for the standard medium. The relative growth indices were as follows:

Without Fe	With Fe (mg.-ion per liter)		
	0.003	1.006 (1 U_{Fe})	0.009
0.65	0.84	1.00	1.14

Neither the chloride nor the citrate appears to have given any considerable promise as a source of iron in cultures of this sort. It is equally clear that $Fe_2(SO_4)_3$ was an excellent source of iron, giving progressively greater growth as the Fe concentration was higher; also that the standard medium

(with 0.006 mg.-ion of Fe per liter) was markedly deficient in Fe for these root-tips. Representative samples of root-tips grown without Fe and with these three different concentrations of $Fe_2(SO_4)_3$ are shown by the photographs of figure 5. It is obvious that the highest Fe concentration tested

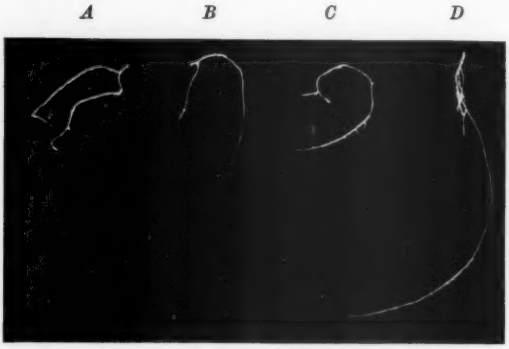

FIG. 5. Representative root-tips grown 2 weeks in media that differed only with respect to the concentration of Fe as $Fe_2(SO_4)_3$: *A*, without Fe; *B*, *C*, and *D*, with 0.003, 0.006 (standard), and 0.009 mg.-ion of Fe per liter respectively. About 0.6 natural size.

(0.009 mg.-ion per liter) gave by far the best growth, while the media with Fe concentration of 0.003 or 0.006 mg.-ion per liter gave relatively little growth, being only slightly superior to the medium without iron.

ANIONS

The effects of omitting any three of the four main anions of the standard medium were tested in a series comprising four modified media besides the standard one. These experiments were carried out at about 25° C., in continuous weak artificial light, and there were 20 cultures for each medium. Each of the four modified media was like the standard one with respect to dextrose, yeast extract, all the cations of the USPENSKI formula, PO_4, and $Fe_2(SO_4)_3$; and each contained, in addition, either NO_3, Cl, SO_4, or CO_3, making in each case the total anion concentration like that of the standard medium. According to the anion that predominated in each, these four modified media may be named the nitrate medium, the chloride medium, the sulphate medium, and the carbonate medium. It will be observed that, aside from possible impurities in the salts used, and aside from the yeast extract used, the nitrate medium contained no Cl or CO_3, and only the very small amount of SO_4 corresponding to 0.006 mg.-ion per liter as $Fe_2(SO_4)_3$; and that neither of the three remaining modified media contained any nitrate, etc. These modified media were prepared according to the specifications for the standard medium but with the following substitutions: For the nitrate

medium $MgNO_3 \cdot 6H_2O$ was used instead of $MgSO_4 \cdot 7H_2O$ and KNO_3 was used in place of K_2CO_3. For the chloride medium $CaCl_2$ was used instead of $Ca(NO_3)_2 \cdot 4H_2O$, $MgCl_2 \cdot 6H_2O$ was used instead of $MgSO_4 \cdot 7H_2O$, and KCl was used in place of both KNO_3 and K_2CO_3. For the sulphate medium $CaSO_4 \cdot 2H_2O$ was used instead of $Ca(NO_3)_2 \cdot 4H_2O$ and K_2SO_4 was used in place of both KNO_3 and K_2CO_3. For the carbonate medium $CaCO_3$ was used instead of $Ca(NO_3)_2 \cdot 4H_2O$, $3MgCO_3 \cdot Mg(OH) \cdot 3H_2O$ was used instead of $MgSO_4 \cdot 7H_2O$, and K_2CO_3 was used in place of KNO_3. Both after autoclaving and at the end of the experiment, all but the carbonate medium showed pH values between 5.4 and 5.7 and no precipitation was observed. The carbonate medium was more alkaline, with a pH value of 5.95, and it showed some precipitation.

The relative values of the growth indices derived from this series are as follows, the actual value for the standard medium being 103 mm.:

Standard medium	1.00
Nitrate medium (with NO_3 and PO_4 but practically without either Cl, SO_4, or CO_3)	0.87
Chloride medium (with Cl and PO_4 but practically without either NO_3, SO_4, or CO_3)	0.73
Sulphate medium (with SO_4 and PO_4 but practically without either NO_3, Cl, or CO_3)	0.55
Carbonate medium (with CO_3 and PO_4 but practically without either NO_3, Cl, or SO_4)	0.32

Root-tips grown in the standard medium were apparently in excellent condition, with an average of 7 branches each. Those grown in the nitrate medium appeared to be in very good condition but they were more slender than those grown in the standard medium; they had, on the average, 6 branches each. Root-tips grown in the chloride medium were generally shorter than those grown in the standard medium or in the nitrate medium, although they had, on the average, 8 branches each; both main root and branches were remarkably thick and stubby, with somewhat swollen tips. Root-tips grown in the sulphate medium were small but their average number of branches was eight and they had much the same appearance as those grown in the standard medium, excepting with respect to size. Those grown in the carbonate medium were clearly in very poor condition, crooked and swollen, and with an average of only 3 branches each, the branches being very short and stubby.

Representatives from these five media are shown in the photograph of figure 6. The fact that every one of the four modified media gave considerable growth indicates that vitality was maintained for the 2-week period in spite of these great chemical modifications of the medium. That the three

modified media without CO_3 all have good, or at least fair, growth, while the carbonate medium gave very poor growth, appears to support the suggestion, already made, that CO_3 might well be omitted from media of this general type for the plant material and period length used in this study. The omission of Cl, SO_4, and CO_3 together (nitrate medium) resulted in a growth value 87 per cent. as large as was obtained with the complete standard medium, from which it appears that Cl and SO_4, as well as CO_3, were unessential to good growth.

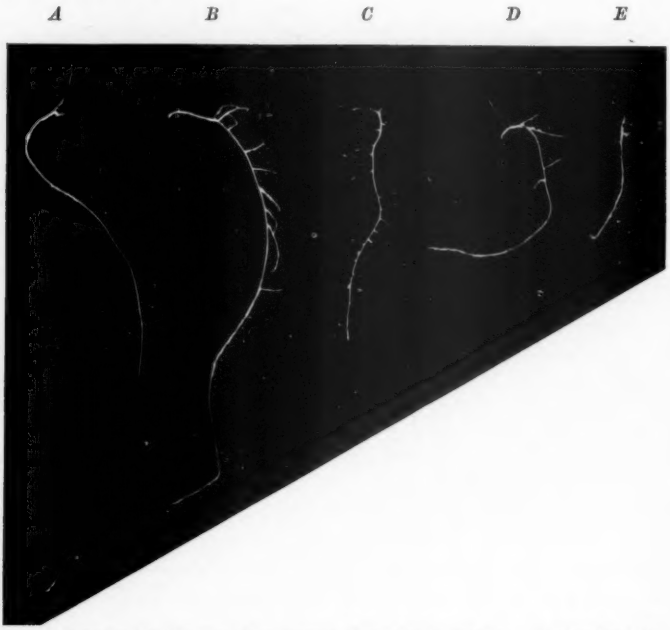

FIG. 6. Representative root-tips grown 2 weeks in media: *A*, standard in all respects; *B*, like *A* but with SO_4 and CO_3 replaced by NO_3; *C*, like *A* but with NO_3, SO_4, and CO_3 replaced by Cl; *D*, like *A* but with NO_3 and CO_3 replaced by SO_4; *E*, like *A* but with NO_3 and SO_4 replaced by CO_3. About natural size.

Of the four anions here considered, NO_3 was obviously the most important for extensive growth, but the good or medium growth obtained with the chloride medium and with the sulphate medium indicates that even NO_3 was not essential for growth in these 2-week tests. Without NO_3 a relative growth index as great as 0.73 was obtained, which is surprising in view of the great importance generally assigned to nitrates in the nutrition of higher green plants, and in view of the generally accepted statement that such organisms are unable to utilize molecular nitrogen (see LIPMAN and TAYLOR

(**4**), however). It is of course logically possible that nitrification occurred in the sulphate medium and in the chloride medium, through the action of nitrifying bacteria, but there is no direct evidence favoring such a supposition. A considerable but surely very small amount of NO_3, may have been introduced into the media planned to be without NO_3, in the yeast extract, and as possible impurities in the dextrose used or in the salts employed. It is also logically if only remotely possible that the original 3-mm. root-tips may have carried a sufficient amount of nitrogenous compounds to maintain good growth for two weeks, but cultures in the chloride medium gave final total lengths about 25 times as great as the original length (3 mm.), and those in the sulphate medium gave final total lengths about 19 times as great as the original length. Root-tips grown in the chloride medium or in the sulphate medium formed, on the average, 8 new growing points (branch roots). If one of these tips 3 mm. long contained sufficient assimilated or assimilable nitrogen to support such pronounced enlargement, and such multiplication of meristematic tissue (without any apparent degeneration of the older and basal tissues), that fact should be noteworthy.

The nitrate ion and the ammonium ion were compared, as to their influence on the growth of these root-tips, by means of a number of experiments with media that were like the standard one in regard to all features excepting the nitrogen source but different from the standard medium and among themselves in that respect. Several concentrations of NO_3 and its omission, and the substitution of NH_4Cl for $Ca(NO_3)_2$ and KNO_3 were thus tested. There were 10 cultures for each N_2 concentration, and cultures were carried out both in continuous darkness and in continuous weak artificial light, the temperature being about 26°. The presence of both NO_3 and NH_4 together

TABLE III

NO_3	NH_4	Total N_2	Relative growth index
mg.-ion per l.	*mg.-ion per l.*	*mg. per l.*	
0.0	0.0	0.0	1.04
0.0	0.32	4.5	0.93
0.0	0.64	9.0	0.98
0.45	0.0	9.8	1.09
0.56*	0.0*	12.0*	1.00
0.0	0.96	13.5	0.95
0.0	1.28	18.0	1.00
0.90	0.0	19.5	1.00
0.0	1.60	22.5	1.01
1.35	0.0	29.3	1.00

* Standard medium.

in the same medium was not tested. All of these tests gave results sensibly like those obtained with standard medium. Table III shows the nature and concentration of the nitrogen source used in ten of these modified media, together with the relative growth index obtained with each modification. With the standard medium (marked by asterisks), excellent growth was obtained and the actual average index was 66.4 mm., which is taken as unity.

In these tests it apparently made no significant difference whether NO_3 or NH_4 was purposely present or absent, or whether either of these nitrogen sources was used at low or at high concentration. This strengthens and extends the conclusions reached from the experimental series previously described.

Effects of modification of the PO_4 concentration of the medium were tested in three series. The temperature varied between 22° and 26°. In two series constituents other than KH_2PO_4 were maintained at the concentrations specified for the standard medium and the PO_4 concentration was 0.0, 0.5, 1.0, or 1.5 U_{PO_4}. There were ten cultures for each of these four media. A third series had the same four PO_4 concentrations, but each of these was tested with eight different pH values, secured by means of small additions of HCl or NaOH. There were three cultures for each of these 32 media, or 96 in all. These experiments showed no pronounced differences in growth, excepting as the PO_4 content of the medium influenced the final H-ion equilibrium, which has been considered in an earlier paper (**10**).

When the SO_4 of the standard medium was partly or wholly replaced by Cl, and also when the concentration of SO_4 was 1.5 U_{SO_4} (Na_2SO_4 being added to the medium in the latter case), growth was sensibly the same as in the unmodified standard medium.

When CO_3 was omitted from the standard medium the resulting modified medium gave greater growth than was obtained with the standard medium itself, and media with CO_3 concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 U_{CO_3} gave progressively less growth as the CO_3 concentration was greater. These statements are based on two special experiments with temperature between 22° and 27°, one with weak continuous artificial light and the other with

TABLE IV

	Without CO_3	With CO_3 (mg.-ion per liter)				
		0.0075	0.0150*	0.0225	0.0295	0.0370
Average indices (mm.)	99.3	88.9	67.8*	52.1	43.0	35.7
Relative indices	1.47	1.31	1.00*	0.77	0.63	0.53
Final pH values	5.05	5.00	5.10*	5.15	5.30	5.40

* Standard medium.

stronger continuous artificial light. The actual and relative growth indices derived from the first of these special experiments are shown in table IV, those for the standard medium being marked with asterisks.

Three representative root-tips from this experiment are shown in figure 7. Those grown in the medium without CO_3 appeared to be in excellent

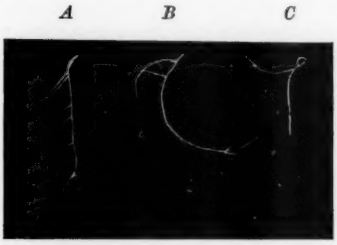

FIG. 7. Representative root-tips grown 2 weeks in media that differed only with respect to the concentration of CO_3: *A*, without CO_3; *B* and *C*, with 0.25 mg.-ion per liter (standard) and 0.50 mg.-ion per liter respectively. About natural size.

condition, with an average of eight branches each. Those grown in the standard medium (with 0.015 mg.-ion of CO_3 per liter) had on the average only five branches each, and those grown in the medium with CO_3 concentration of 2.0 U_{CO_3} were short and stubby and had an average of only two branches.

A repetition of this experiment gave essentially the same results. These series thus support the conclusions indicated by previous experiments with CO_3; namely, that the presence of CO_3 in the medium tended to have a retarding effect on the development of root-tips grown therein. But it is to be noted that the culture series represented by figure 4 indicated considerably less growth (relative index value of 0.82) when CO_3 was omitted than was given by the unmodified standard medium.

This retarding influence of CO_3 as indicated by these experiments, can hardly be attributed directly to low H-ion concentration, for the final pH values were found to be nearly the same whether CO_3 was present or not, and whether its concentration was high or low, as is seen in table IV. It is perhaps possible that the retarding influence may have been related to Fe supply, as suggested by USPENSKI (**8**); but it is surprising that these root-tips should have been injured or retarded in their growth by such low concentrations of CO_3, an ion that must be generally present at considerable concentrations in rapidly growing tissues in which photosynthesis is not active.

Summary

1. In a preliminary study reported previously (**10**), it was found that 3-mm. root-tips excised from wheat seedlings grew excellently for a 2-week

period and without renewal of the liquid culture medium when the inorganic salt contents of the latter were like those specified for the USPENSKI solution (8), and when each liter of medium contained also 20 gm. of dextrose and extract from 0.1 gm. of dry brewers' yeast. Within the limits of that study, the medium thus characterized gave best growth under the following conditions: (1) when its pH value was about 5.0–5.5; (2) when each culture flask, with its single root-tip, contained not less than 25 ml. of medium; (3) when the culture temperature was maintained about 26°–27°; and (4) when the cultures received continuous electric illumination from Mazda "daylight" bulbs. In the present paper are presented results from additional preliminary experiments with root-tips like those used before, in which the experimental variables were the ion concentrations of the culture medium, aside from H-ion and OH-ion concentrations. The medium just described was taken as standard, being used for control cultures, and numerous modifications of it (with respect to inorganic ion concentrations) were tested, generally under the conditions just specified for pH value, volume of medium, temperature, and illumination. The main results may be summarized as follows.

2. The standard medium was notably improved by lowering its Ca concentration by 50 per cent., but its effectiveness was lowered by raising the Ca concentration or by omitting Ca. Raising or lowering the K concentration or omitting K gave poorer growth than was given by the unmodified standard medium. Of 16 systematically chosen combinations of Ca concentration and K concentration (including the omission of one or both of these ions as well as the raising or lowering of their concentrations), only one combination (lowering the Ca concentration by half and keeping the K concentration unmodified) was superior to the one specified for the standard medium, and a single other combination (lowering the Ca concentration and raising the K concentration, each by 50 per cent.) gave an average growth value equaling but not exceeding the corresponding value given by the unmodified standard medium. The remaining tested combinations of these two concentrations were all inferior to the standard.

3. The standard medium was somewhat improved by the omission of Mg and its effectiveness was lowered by raising the Mg concentration. But when CO_3 was omitted and the Mg concentration was raised by 50 per cent., the effectiveness of the resulting modified medium equaled or slightly exceeded the effectiveness of the unmodified standard medium. In one test, simply omitting CO_3 led to somewhat poorer growth than was given by the standard medium; but in two special experiments on the influence of CO_3 concentration, this omission led to very marked improvement of the medium.

4. The standard medium was markedly improved by raising the concentration of $Fe_2(SO_4)_3$ by 50 per cent., and its effectiveness was lowered

by the omission of that salt or by lowering its concentration. Ferric chloride and "ferric citrate" were each found to be unsatisfactory as sources of Fe in these experiments.

5. Omitting NO_3 or raising or lowering its concentration in the medium exerted no significant effect on growth, and the use of NH_4 instead of NO_3, at various concentrations, was also without considerable influence; it made no difference whether NO_3 or NH_4 was used as nitrogen source, or whether either of these ions was at low or at high concentration. It is surprising that excellent growth was obtained in these 2-week cultures, without any nitrogenous substance in the medium excepting such very low concentrations as may have been due to impurities in the salts and dextrose used or in the yeast extract, which was present in all media.

6. Omitting SO_4 or raising or lowering its concentration in the medium did not show any influence on growth, and the same is true for PO_4, excepting as PO_4 concentration affected the pH value of the medium.

7. The standard medium was improved by the omission of CO_3, or at least the effectiveness of the medium was not greatly reduced by that omission. Raising the CO_3 concentration lowered the effectiveness of the medium.

8. Addition to the standard medium of a considerable concentration of Cl or Na was without notable effect on growth; one or the other of these ions, neither of which was used in the standard medium, was added in the preparation of some of the modified media discussed.

These experiments were carried out at the Boyce Thompson Institute for Plant Research while the writer held a National Research Fellowship in Botany. This account has been prepared for publication at the Laboratory of Plant Physiology of the Johns Hopkins University, with critical help from Professor BURTON E. LIVINGSTON.

ROCKEFELLER INSTITUTE OF MEDICAL RESEARCH
PRINCETON UNIVERSITY
PRINCETON, N. J.

LITERATURE CITED

1. BEILSTEIN, F. K. Handbuch der organischen Chemie. IV Auflage. Erstes Ergänzungswerk. Julius Springer, Berlin. 1932.
2. BELLONI, E. I. Sali organici del ferro. II. Citrati ferrici e citrati ferrici ammoniacali. Gaz. Chim. Ital. **50**^{II}: 159–212. 1920.
3. HOPKINS, E. F. Iron-ion concentration in relation to growth and other biological processes. Bot. Gaz. **89**: 209–240. 1930.
3a. ————. (private communication to the writer). 1931.
4. LIPMAN, C. B., and TAYLOR, J. K. Do green plants have the power of fixing elementary nitrogen from the atmosphere? Jour. Franklin Inst. **198**: 475–506. 1924.

5. MORITA, K., and LIVINGSTON, B. E. Some solution cultures of wheat without potassium. Bot. Mag. Tokyo **34**: 71–90. 1920.
6. Pharmacopoeia of the United States of America. VIIIth ed. P. Blakiston's Son & Co., Philadelphia. 1905.
7. SHIVE, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.
8. USPENSKI, E. E. Eisen als Faktor für die Verbreitung niederer Wasserpflanzen. Pflanzenforschung herausgegeben von R. KOLKWITZ. G. Fischer, Jena. 1927.
9. ————, and USPENSKAJA, W. J. Reinkultur und ungeschlechtliche Fortpflanzung des *Volvox minor* und *Volvox globator* in einer synthetischen Nährlösung. Ztschr. Bot. **17**: 273–308. 1925.
10. WHITE, P. R. Influence of some environmental conditions on the growth of excised root tips of wheat seedlings in liquid media. Plant Physiol. **7**: 613–628. 1932.

ACIDITY AND ALKALINITY PRODUCED BY CHANGES IN THE NITROGEN, SULPHUR, AND CARBON CYCLES[1]

JOHN P. CONRAD

(WITH FOUR FIGURES)

Introduction

Culture media, whether they be soils, nutrient solutions for higher plants, or bacteriological cultures, are subject to fluctuations, often of considerable magnitude, in their H-ion concentrations. Plant and animal fluids and contents of living cells also fluctuate in reaction. One phase of the problem upon which the writer is working involves changes in the reaction of soils, apparently due in part to the changes in the nitrogen cycle. It seemed desirable to investigate in a theoretical way the principal changes that could arise from these and other transformations. The results secured as well as the methods used offer the possibility of wider application than to this specific problem, being perhaps most applicable to the fields of plant physiology, soil chemistry, and bacteriological chemistry. For that reason, they have been prepared for publication.

Certain changes in the nitrogen cycle produce acidity, other changes produce alkalinity, and still other changes in the nitrogen cycle have little or no effect on the pH of the medium. Many of these changes have been worked out quantitatively, others only qualitatively.[2] Confusion exists in some plant and soil literature bearing on these changes. This paper is an attempt to develop by theoretical reasoning, from the fields of inorganic and biological chemistry, a quantitative expression of the acidity and alkalinity produced by changes in the nitrogen cycle, culminating in a chart which shows these relations graphically. Some attention will be given also to production of acidity and alkalinity resulting from transformations within the carbon and sulphur cycles. A subsequent paper will give experimental data bearing on a part of this cycle.

It will be convenient in the following development to speak of the production of one equivalent of "acidity" as meaning also the disappearance of one equivalent of alkalinity, or a fraction of each, such that the sum of the fractional equivalents of acid produced and alkalinity disappearing will equal one; and conversely, with the production of one equivalent of alkalinity.

[1] Contribution from the Division of Agronomy, University of California, Davis, California.

[2] Citations to much of the literature bearing on this point will be reserved for later publication.

As acidity and alkalinity are measured by titrations, it is necessary to have a datum or end-point for reference. I have selected as the reference in the first part of this theoretical development a pH of 4.3, but shall extend the range both to the acid and considerably to the alkaline side of this point. This point was chosen because it represents the pH of the solution used as reference in the methyl orange titrations later employed, and is not far also from the iso-electric point of many of the proteins. The CO_2 of the atmosphere does not influence titrations at this pH.

The cycle: Elementary nitrogen, ammonia, and nitric acid

Since many factors, such as the other chemicals used in the media, the products of other reactions, the incompleteness of the chemical change involving nitrogen, etc., may somewhat complicate the titrations of the original materials and the final end-products of the reaction, it will be simpler to take transformations from the realm of inorganic chemistry in so far as possible. For purposes of comparison, an analogy between heat and acidity will be used. Changes in the amount of calorific heat in a system may arise from at least three different factors:

A. By addition or withdrawal of heat from the system, *i.e.*, by changing the temperature but not the state of the system.
B. By physical transformations within the system (such as liquid to vapor, etc.).
C. By chemical transformations within the system itself.

Similarly, titrable acidity (positive or negative) may change in a system:

A. By addition of acid to or its withdrawal from the system.
B. By physical transformations within the system.
C. By chemical transformations within the system.

The first is easy to understand and the second may occur, but probably not so prevalently with acidity as with heat. Considering factors *A* and *B* as constant in the discussion following, the effect of chemical transformations will be considered; as, for instance, a chemical reaction of great industrial importance: $S + \frac{3}{2}O_2 + H_2O = H_2SO_4 + 103{,}240$ calories.[3]

The heat of this reaction is invariable no matter in how complex a system it may take place, as long as the initial and the final state points are the same. If in a complex system a different heat of reaction is obtained experimentally, this is evidence that some other reaction or reactions have taken place. Likewise if the reactants for this equation could be titrated to an end-point at pH 4.3, and the end-products titrated to the same pH, a change

[3] By computation from heats of formation of H_2O and H_2SO_4 given by HODGMAN and LANGE (7).

of two equivalents of titrable acidity for each gram-atom of sulphur transformed will be secured. This reaction will always give these results no matter in how complex a system it takes place, provided the initial and final state points are the same and the end-point of titration is above pH 4. This must be true or the law of the conservation of matter would be violated.

As with the heat of reaction so also with the titrable acidity of this reaction, it is immaterial whether the reaction occurs all at once or by stages.

If the sulphur is used up in this reaction and it is known to be oxidized according to the reaction given, the change resulting in the increase of sulphate-ion is directly proportional to the heat evolved and also to the change in titrable acidity resulting from *this reaction.* Since in this development physical conditions are considered as being kept constant, and no additions of heat or titrable acidity are being made, no heat can be evolved nor titrable acidity arise except through chemical transformations.

From the preceding analogy, it follows that changes in the amount of titrable acidity (considering alkalinity as negative acidity) are equal to the algebraic sum of the titrable acidities arising from each of the reactions taking place. In other words, in any system the total changes in titrable acidity (factors A and B remaining constant) are equal to the algebraic sum of the changes due to each individual reaction. It is the titrable acidity arising from each of many of the possible reactions within the nitrogen, sulphur, and carbon cycles which will be considered.

Elementary nitrogen to ammonia

A chemical process recently coming into great commercial prominence is the Haber-Bosch method for making gaseous ammonia. The reaction generally ascribed to this process is as follows:

$$N_2 + 3\ H_2 = 2\ NH_3.$$

Since the reactants are not titrable with acid or alkali and therefore have a zero titration value, and since the NH_3 if dissolved in water may be titrated, requiring 1 mol of acid for each mol of NH_3, then for each gram-atom of nitrogen fixed as ammonia one[4] equivalent of base (in this case ammonia) is produced. Thus a change of one equivalent of titrable alkalinity is produced for each gram-atom of nitrogen transformed from N_2 to NH_3, no matter whether the action goes on in the Haber-Bosch process, or in nature through the fixation of nitrogen by a legume and the subsequent decay of this crop's residues to form NH_3.

Elementary nitrogen to nitric acid

In the arc method of fixing gaseous nitrogen the process takes place in several steps, but a chemical reaction which would express the original materials and the final product is as follows:

[4] Theoretically it may be slightly less than one, as NH_3 is a weak base.

$$2\ N_2 + 2\ H_2O + 5\ O_2 = 4\ HNO_3.$$

The initial material is considered to have zero titration value, but with the end-product of the process, nitric acid, one equivalent of acid is produced for each gram-atom of nitrogen changed.

Ammonia to nitric acid

The transformation of ammonia to nitrate in the soil now has a counterpart in industry known as the Ostwald process (**11**). By the use of a platinum catalyst and in several steps, the ammonia gas is transformed to nitric acid by the following over-all reaction:

$$NH_3 + 2\ O_2 = HNO_3 + H_2O.$$

On titration, the chemicals on the left side of the equation give one equivalent of base, and those on the right one equivalent of acid, for each gram-atom of nitrogen transformed. When nitrogen is changed from ammonia to nitric acid there is a net change of titrable acidity equal to two equivalents of acid for each gram-atom of nitrogen transformed.

If NH_3 is neutralized by sulphuric acid to form $(NH_4)_2SO_4$ and the nitrogen in the ammonium salt oxidized to nitrate, there will be present the original sulphuric acid and the nitric acid formed. In this transformation for each mol of ammonium sulphate there will be produced four equivalents of acid. As there are two gram-atoms of nitrogen per mol of ammonium sulphate, there is an increase of two equivalents of titrable acidity for each gram-atom of nitrogen transformed.

Elementary nitrogen to nitrous acid

When the gases in the arc process are dissolved in water, nitrous acid is formed according to the reaction:

$$2\ N_2 + 2\ H_2O + 3\ O_2 = 4\ HNO_2.$$

Thus for each gram-atom of nitrogen transformed from elementary nitrogen to nitrous acid, one equivalent of titrable acidity is produced except as will be brought out later.

Ammonia to urea

If ammonium carbonate is heated under appropriate conditions, urea results as follows:

$$(NH_4)_2CO_3 = (NH_2)_2CO + 2\ H_2O.$$

Titrating the initial $(NH_4)_2CO_3$ we get an equivalent of alkalinity for each gram-atom of nitrogen, while practically urea, because it is a very weak base, gives none, except as will be brought out later when the acidity of the system is high.

Other nitrogen transformations

From the preceding, we would predict that the transformation of nitrates back to N_2 would produce titrable alkalinity, and that of NH_3 to N_2 produce titrable acidity. As these reactions do not normally occur in inorganic chemistry, some reactions involving the formation of N_2 from nitrogen salts may be investigated. If the alkalinity produced by the formation of NH_3 from the union of N_2 and H_2 is neutralized by nitrous acid, ammonium nitrate is produced. On titrating, this will be practically neutral. But ammonium nitrite decomposes on heating to form elementary nitrogen and water as:

$$NH_4NO_2 = N_2 + 2\ H_2O.$$

The final products are not titrable with acid or alkali and hence have a zero titration value. Very little or no change of titrable acidity is brought about by this reaction.

On the other hand, if the alkalinity of ammonia is neutralized with nitric acid, ammonium nitrate is formed, which has for many practical purposes no titrable acidity. But NH_4NO_3 decomposes on heating to give nitrous oxide (N_2O) and water, according to the following reaction:

$$NH_4NO_3 \longrightarrow N_2O + 2\ H_2O.$$

If nitrous oxide is dissolved in water, a very weak acid, weaker than carbonic, is formed, $H_2N_2O_2$. This acid is called hyponitrous acid. Only at high pH values would its acidity become manifest. Figure 1 illustrates graphically these relationships.

Except as will be brought out later, N_2, urea, ammonium nitrite, and ammonium nitrate are for most practical purposes neutral in reaction.

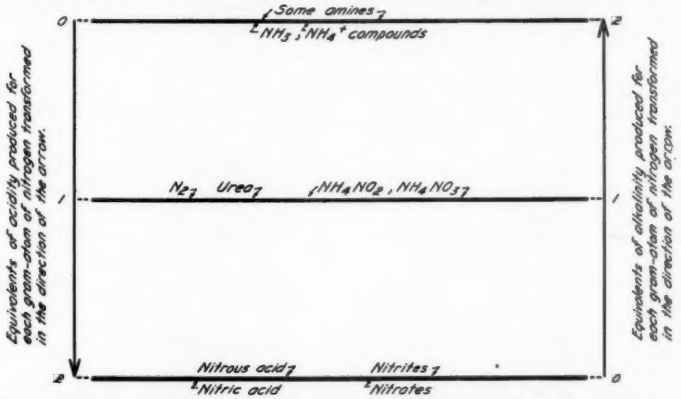
Some amines
NH3 ; NH4+ compounds
N2 Urea NH4NO2 , NH4NO3
Nitrous acid Nitrites
Nitric acid Nitrates
Equivalents of acidity produced for each gram-atom of nitrogen transformed in the direction of the arrow.
Equivalents of alkalinity produced for each gram-atom of nitrogen transformed in the direction of the arrow.
0
1
2

FIG. 1. Titrable acidity and alkalinity produced by changes in nitrogen cycle. Very little error arises in using this chart between pH values of 4.5 and 8 (see fig. 2).

Transformation of one gram-atom of nitrogen from any or all of the preceding substances to ammonia will result in the production of one equivalent of alkalinity. Likewise the transformation of one gram-atom of nitrogen from this group of substances to nitrate or nitric acid will result in the production of one equivalent of titrable acidity, no matter in how complex a system it takes place nor in how many stages, subject of course to the condition that factors *A* and *B* remain constant.

Transformations of protein nitrogen

From solution-culture and field studies, some details of which will be taken up in a subsequent paper, it is apparent that the medium becomes more acid when ammonium salts of strong acids, with the exception of NH_4NO_3, are used as a plant nutrient; also the medium becomes more alkaline when nitrates, except NH_4NO_3, are used. On the average, therefore, the compounds of nitrogen in the plant must have a position on the chart somewhere intermediate between NH_3 on the one hand and HNO_3 on the other. Proteins are undoubtedly the chief class of nitrogenous compounds in normal plants, and therefore could be expected to lie on the chart somewhere between ammonium compounds and nitrates.

A more direct means of determining the position of a given protein lies (1) in a consideration of its titration curve, and (2) in a consideration of its iso-electric point in so far as that might represent the pH of a suspension of the pure protein in water. This assumption is on the basis of LOEB'S work (**8**, p. 35). In soils, MATTSON (**9**) has shown that when the iso-electric point is not at neutrality, some differences may arise between the two values.

A few proteins will be considered as typical of all proteins. Discrepancies arising because any protein does not behave as do the types are, it is believed, of relatively minor importance in this connection. Some of the proteins selected are not found in plants, but because the titration curve and elementary composition of each are known, they are included. Those chosen, and the properties of each applicable to the present problem, are given below:

Protein	Chemical elements present	pH of aqueous suspension	Investigators cited
Gelatin	C, H, O, N	4.7	LOEB (**8**, figs. 7, 13)
Durumin	C, H, O, N, S	5.9	HOFFMAN and GORTNER (**6**, table 78)
Teozein	C, H, O, N, S	5.2	" (**6**, table 79)
Fibrin	C, H, O, N, S	4.9	" (**6**, table 81)
Egg albumin	C, H, O, N, S, P	4.8	LOEB (**8**, figs. 4, 11)
Casein	C, H, O, N, S, P	4.7	LOEB (**8**), HOFFMAN and GORTNER (**6**, table 80)

If we completely oxidize each of these proteins in turn with oxygen, dissolve the end-products of the reaction in water, and titrate them to our reference pH of 4.3, in equilibrium with the atmosphere, the following results are secured:

In the original protein each gram-atom of	produces one mol each of the acid below	which titrated at pH 4.3 gives the number of equivalent of acidity below	Remarks
C	H_2CO_3	0	lost to the air
N	HNO_3	1	almost fully neutralized
S	H_2SO_4	2	"
P	H_3PO_4	1	only 1/3 neutralized

The nitrogen transformation from the protein, gelatin, at its iso-electric point to HNO_3 titrated at pH 4.3 would produce one equivalent of acidity. Gelatin may then be placed tentatively with N_2 on the chart. But it required (**8**, fig. 7) about 1.5 cc. of 0.1 N HCl or H_2SO_4 to bring 1 gm. of purified gelatin in 100 cc. of H_2O from its iso-electric point of pH 4.7 to 4.3, our reference point in titration. One gm. of gelatin assumed to contain 17.80 per cent. nitrogen fully oxidized would give 127 cc. of 0.1 N HNO_3. The error then in placing gelatin with N_2 on the chart at pH 4.3 would be approximately 1.5 divided by 127, or 1.18 per cent. Considering the HNO_3 formed as a measure of the nitrogen transformation alone (the H_2SO_4 and H_3PO_4 formed being credited to sulphur and phosphorus transformations) the errors involved in placing each of the proteins mentioned at N_2 on the chart can be computed in a similar manner. These are for durumin about 0.7 per cent.; for teozein, 0.1 per cent.; for fibrin, 0.3 per cent.; for egg albumin, 0.8 per cent.; and for casein, 0.5 per cent.

It would be desirable to know the errors involved at other pH reference points. If the titration end-point be pushed up to the alkaline side soon a place is reached where no end-point can be secured in equilibrium with the atmosphere because of the CO_2. We can then assume titration of the original gelatin and the products of its complete oxidation in a CO_2-free atmosphere. Under such conditions, the CO_2 produced by the oxidation would be eliminated and the difference would be due to the nitrogen change alone.

In titrating 0.8 gm. of purified gelatin in 100 cc. at a pH of 8.4 (about the phenolphthalein end-point), nearly 2.9 cc. of 0.1 N NaOH is required (**8**, fig. 13). The same amount of gelatin fully oxidized in equilibrium with a CO_2-free atmosphere would require 101.5 cc. of the same strength of NaOH to neutralize it. At pH 8.4, then, an error of about 2.9 per cent. results from placing gelatin at the position of N_2 on the chart.

By arbitrarily attributing all of the error involved in the titration curve of each protein as due to the nitrogen change alone, the errors involved in placing these proteins at pH 8.4 at the position of N_2 on the chart are for durumin, 0.4 per cent.; for teozein, 0.4 per cent.; for fibrin, 4.0 per cent.; for egg albumin, 3.3 per cent.; and for casein, 7.0 per cent.

Assuming no error from the titration curve as due to sulphur and phosphorus changes, each gram-atom of sulphur in the protein would result in practically two equivalents of titrable acidity at pH 8.4 due to complete oxidation of the protein, while each gram-atom of phosphorus would likewise result in about two equivalents of titrable acidity (H_3PO_4 is about 2/3 neutralized at pH 8.4).

With the exception of gelatin, each of the proteins being considered results, with complete oxidation, in acidity in excess of that due to the nitrogen change. Acidity arising from the H_2SO_4 and H_3PO_4 must be taken into consideration.

With the definite placing of proteins on the nitrogen chart it is clearly seen that the transformation from nitrates to proteins will result approximately in one equivalent of titrable alkalinity for each gram-atom of nitrogen changed. At the same time additional alkalinity will result from the sulphur transformation from sulphate to protein. Though phosphorus is normally not a constituent of plant proteins, it may enter into compounds whose titration curves are similar. Additional alkalinity would then result. Any deviation from this would be contrary to the law of the conservation of matter, and therefore invalid.

Although it would be desirable to have the pH of a suspension or solution of the pure protein in pure water as the best experimental evidence at hand, such a pH may be secured indirectly by assuming that such a suspension is at, or very near, the iso-electric point of the protein in question. Assuming again that other methods of securing the iso-electric point will give indirectly the pH of a water suspension of the pure protein, the iso-electric point of some other proteins is of interest.

Thus CSONKA, MURPHY, and JONES (5) report iso-electric points of five albumins from pH 4.2 to 5.5, thirty globulins from pH 4.5 to 5.5, and five prolamines from pH 5.9 to 6.6. CSONKA and JONES (4) report two glutelins of wheat and one each of rice, oats, and corn as having iso-electric points of pH 6.45. PEARSALL and EWING (10) report the iso-electric points of some plant proteins as found by the precipitation methods. These varied from pH 3.2 to 5.6. Excluding three extremes, the rest varied but little from pH 4.5. CHIBNALL (3) reports iso-electric points of leaf-cytoplasmic proteins from pH 3.5 for rhubarb to pH 5.1 for broad bean, with an average near pH 4.4. Other iso-electric points have been determined by others, but

these in general fall within the range already given, with the possible exception of glutenin with an iso-electric point of 7.0, reported by TAGUE (**13**).

It is evident that many if not most of the proteins have iso-electric points close to those here presented whose titration curves are available. These observations allow us then to place proteins at the same place on the chart as N_2, considering the errors presented almost negligible for many purposes.

Table I lists some of the nitrogen transformations and the titrable acidity and alkalinity resulting from each at usual pH values.

TABLE I

NITROGEN TRANSFORMATIONS	NET PRODUCTION IN EQUIVALENCES OF ACIDITY AND ALKALINITY PER GRAM-ATOM OF NITROGEN TRANSFORMED	
	ACIDITY	ALKALINITY
Nitrogen fixation by legumes and bacteria to form proteins	0	0
Urea to proteins; proteins to urea	0	0
Assimilation of NH_3 by plants (including lower forms) to form proteins	1	−1
Nitrification of urea, proteins, etc. (net effect)	1	−1
Nitrification of NH_3 and NH_4 compounds	2	−2
Assimilation of nitrates (HNO_3) by plants to form proteins	−1	1
Denitrification (nitrates to N_2)	−1	1
Reduction of nitrates to ammonium compounds (**14**, p. 182)	−2	2

Some of the work done on denitrification is of interest in connection with table I. Alkalinity is produced when nitrates are denitrified and elementary nitrogen is produced. BURRI and STUTZER (**2**), in their work on denitrifying bacteria, found that the cultures rapidly developed alkalinity as the fermentation progressed. Although no figures are given, they found higher alkalinities to develop than could be attributed to decomposition of the nitrates. This increase they attributed to the transformation of some of the alkali citrates used in the media to alkali carbonates, and to the utilization of some more of the citrate radicle as a source of carbon by the organisms.

By causing denitrification to take place with $NaNO_3$, and especially with $Sr(NO_3)_2$, SACHAROWA (**12**) has carried the pH in one step to 8.5 and in another step from 8.5 to 9.4. No figures are available so far as we know to determine experimentally a quantitative relationship between the alka-

linity produced and the nitrogen transformed. According to our chart, for each gram-atom of elementary nitrogen formed there should be one equivalent of alkalinity produced.

An experiment performed by BRIOUX (**1**) is also of interest in the light of the relations shown in figure 1. Using 0.5 gm. of urea in 500 gm. of soil having an initial pH of 6.45, he secured a pH of 8.5 in 48 hours, with a heavy production of ammonia as shown by Nessler's reagent; and after 76 days the pH had changed to 5.35 with the transformation of ammonia to nitrate. Our chart would have qualitatively predicted these results.

Weak nitrogenous acids and bases

Nitrogen transformations given in figure 1 that take place between pH 5 and pH 8 follow the chart very closely and hence undoubtedly apply to most culture media. On account of the hydrolysis of weak acids and bases, however, errors of considerable magnitude may creep in where the pH is beyond these limits.

Let us consider the dissociation of the weak acid H A.

$$H\,A = H^+ + A^- \qquad \text{Then}$$

$$\frac{[H^+]\ [A^-]}{[H\,A]} = K_a$$

$$\log [H^+] + \log \frac{[A^-]}{[H\,A]} = \log K_a \qquad \text{But } \log [H^+] = -pH$$

$$\log \frac{[A^-]}{[H\,A]} = \log K_a + pH \qquad \text{.....(1)}$$

In the case of a definite weak acid, K_a is known. Then for a definite pH the ratio $[A^-]$ to $[H\,A]$ is fixed and can be calculated. If the system be analyzed for $[A^-]$ the undissociated $[H\,A]$ is secured also. With the sum of $[A^-] + [H\,A]$ known and their ratio $[A^-]/[H\,A]$ calculable, the concentration of A^- may be computed.[5] With a transformation in which H A is formed from some other substance (as for example HNO_2 from N_2 in figure 1), the change in the number of equivalents of H^+ which resulted from the transformation is equal to the change in the number of equivalents of A^- produced by that transformation. The transformation affects the titrable acidity and in any but a highly buffered system this will change the pH, sometimes materially. The amount of such change in pH is dependent upon the buffer characteristics of the particular system. A transformation

[5] Other undissociated molecules or complex ions in equilibrium with A^- would lead to error by the use of this procedure, although the general theory is not invalidated thereby.

which would cause a great change in pH in a poorly buffered system would cause a very much smaller change in pH in a highly buffered system. Further consideration of this phase of the problem is beyond the scope of this paper. It is obvious that only the H^+ dissociated from the total H A formed is effective upon the pH of the system or in titration. The fraction of the total H A dissociated is

$$\frac{[A^-]}{[HA]+[A^-]} = \frac{\frac{[A^-]}{[HA]}}{\frac{[HA]+[A^-]}{[HA]}} = \frac{\frac{[A^-]}{[HA]}}{1+\frac{[A^-]}{[HA^-]}},$$

i.e., the fraction dissociated is equal to the ratio $\frac{[A^-]}{[HA]}$ divided by that ratio plus one.

For HNO_2 we have by substitution in equation (1)

$$\log\frac{[NO_2^-]}{[HNO_2]} = \log(4\times10^{-4}) + pH.$$

In table II are given the values of the ratio $\frac{[NO_2^-]}{[HNO_2]}$ and the decimal fraction dissociated for given values of pH.

TABLE II

CALCULATION OF THE FRACTION OF HNO_2 DISSOCIATED AT DIFFERENT pH VALUES; DISSOCIATION CONSTANT OF $HNO_2 = K_a = 4\times10^{-4}$

pH	$\log\frac{[NO_2^-]}{[HNO_2]}$	$\frac{[NO_2^-]}{[HNO_2]}$	FRACTION DISSOCIATED
6.0	2.6	400	0.997
5.0	1.6	40	0.975
4.0	0.6	4	0.80
3.5	0.1	1.26	0.557
3.4	0.0	1.0	0.50
3.0	$\bar{1}.6$	0.4	0.286
2.0	$\bar{2}.6$	0.04	0.038
1.0	$\bar{3}.6$	0.004	0.004

Thus at pH 3.4 each gram-atom of nitrogen transformed to HNO_2 will result in but one-half of an equivalent of titrable acidity, and at pH 2.0

only 0.038 equivalent of titrable acidity, while at pH 4.0 there will be 0.80 equivalent.

A weak base dissociates into $BOH \rightleftarrows B^+ + OH^-$ and $\frac{[B^+]\ [OH^-]}{[BOH]} = K_b$

$$\log [OH^-] + \log \frac{[B^+]}{[BOH]} = \log K_b$$

But $\log [OH^-] = pH - 14$ (approximately at 25°C.)

Then $\log \frac{[B^+]}{[BOH]} = \log K_b + 14 - pH.$

The dissociation constant of NH_4OH is 1.8×10^{-5}, and of urea is 1.5×10^{-14}. These data permit the setting up of similar tables for the fractions dissociated at different pH values for these substances (fig. 2). A typical protein, durumin, is also included from the titration curves mentioned earlier (from data of HOFFMAN and GORTNER).

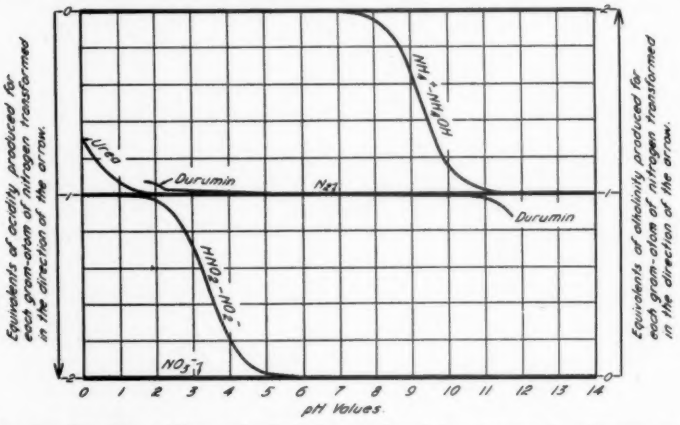
Equivalents of acidity produced for each gram-atom of nitrogen transformed in the direction of the arrow.
Equivalents of alkalinity produced for each gram-atom of nitrogen transformed in the direction of the arrow.
Urea
Durumin
Nil
Durumin
$NH_4^+ \rightarrow NH_4OH$
$HNO_2 \rightarrow NO_2^-$
$NO_3^- \rightarrow$
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH Values

FIG. 2. Titrable acidity and alkalinity produced by changes in nitrogen cycle at different pH values. Proteins represented by durumin, the curve of which is plotted from data of HOFFMAN and GORTNER (6); other proteins have different curves.

When weak acids and bases are involved in these charts, the pH of the system is of very great importance in determining the magnitude of the changes in acidity. In a poorly buffered system the transformations may cause marked changes in pH. For these conditions the chart gives with considerable accuracy the amount of strong acid or base which must be added to keep the system at a constant pH.

It is believed that the number of transformations given here may be extended by the simple expedient of using or finding the titration curves or dissociation constants of the bases and acids not shown. Thus other

proteins, the various amino acids, plant alkaloids, etc., may be added. It is realized that a small source of error may arise because of the use of the concentration formulas rather than of a consideration of the activity of the various ions involved. It is believed, however, that this development together with the charts will give a general picture of the results of these transformations sufficiently accurate for many purposes.

Sulphur cycle

In biology, transformations of sulphur are also important. As is well known, these may influence the H-ion concentration of the medium. From a consideration of the dissociation constants of the various acids of sulphur, similar tables and a chart may be set up. These acids dissociate in two stages, each with a different constant. The values given by HODGMAN and LANGE (7) are as follows:

	CONSTANTS FOR	
ACID	FIRST HYDROGEN	SECOND HYDROGEN
H_2S	9.1×10^{-8}	1.2×10^{-15}
H_2SO_3	1.7×10^{-2}	5×10^{-6}
H_2SO_4	.....	2×10^{-2}

Figure 3 shows the effect of these transformations on the titrable acidity

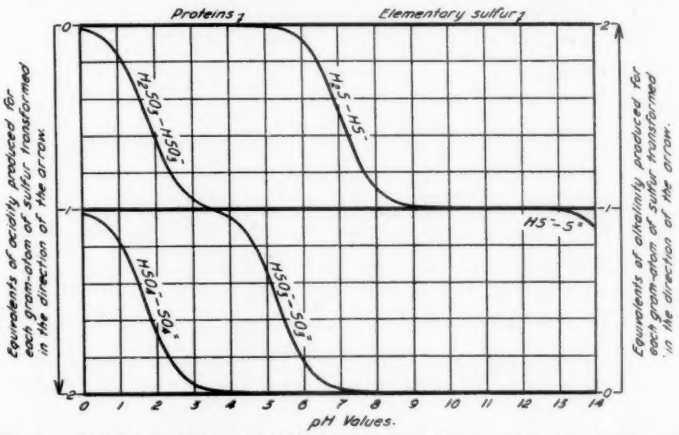
Proteins
Elementary sulfur
Equivalents of acidity produced for each gram-atom of sulfur transformed in the direction of the arrow.
Equivalents of alkalinity produced for each gram-atom of sulfur transformed in the direction of the arrow.
H2S-HS-
H2SO3-HSO3-
HS-S°
HSO4-SO4=
HSO3-SO3=
pH Values.

FIG. 3. Titrable acidity and alkalinity produced by changes in sulphur cycle at different pH values.

at various pH values. Since the values for the titration curves of the proteins have been put into the nitrogen chart, the proteins in the sulphur chart are kept at neutrality through all of the pH values. It is interesting to note that a transformation of a gram-atom of sulphur as sulphide to

sulphate at pH 10 gives but one equivalent of titrable acidity, while the same transformation at pH 4 will result in two such equivalents. At pH 10 the sulphides occur as BHS and the transformation is then to $BHSO_4$, which at that pH is fully effective as a mixture of B_2SO_4 and H_2SO_4; while at pH 4 the sulphide ion occurs practically undissociated as H_2S and this is transformed to H_2SO_4, which at that pH is practically completely effective as a fully dissociated acid.

Carbon cycle

As is well known, transformations within the carbon cycle influence the pH of the culture medium. The dissociation constants of many organic acids are available, but only a few will be considered here in order to reduce the confusion of the chart to a minimum. The dissociation constants of these from HODGMAN and LANGE are as follows:

ACID	DISSOCIATION CONSTANTS FOR FIRST HYDROGEN	SECOND HYDROGEN
H_2CO_3	3×10^{-7}	6×10^{-11}
Acetic CH_3COOH	1.86×10^{-5}	
Formic HCOOH	2.14×10^{-4}	

As compounds of carbon, the sugars, alcohols, and proteins are considered as not contributing to the titrable acidity or alkalinity. It is true that the proteins have a titration curve, but these contributions of the proteins to titrable acidity are for convenience attributed to the nitrogen cycle. Figure 4 brings out some of the relationships in the carbon cycle.

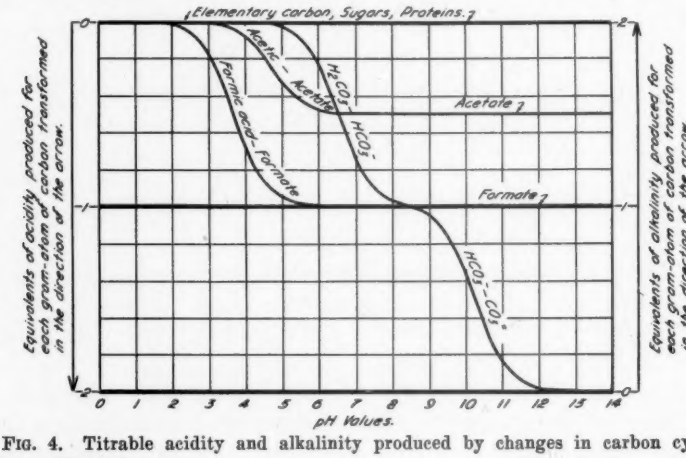
Elementary carbon, Sugars, Proteins.
Acetic → Acetate
Formic acid → Formate
H_2CO_3 → HCO_3^-
Acetate
Formate
$HCO_3^- \rightarrow CO_3^=$
Equivalents of acidity produced for each gram-atom of carbon transformed in the direction of the arrow.
Equivalents of alkalinity produced for each gram-atom of carbon transformed in the direction of the arrow.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH Values.

FIG. 4. Titrable acidity and alkalinity produced by changes in carbon cycle at different pH values.

Because of the weak acids involved, some interesting points are brought out. In a highly buffered system, one gram-atom of carbon as sucrose transformed to H_2CO_3 at pH 12 or 13 will cause about two equivalents of titrable acidity to appear; at pH 8 to 9, about one equivalent of acidity; and at pH below 4.5, practically no change in titrable acidity.

The change of sugars to acetic acid, which has two gram-atoms of carbon per mol, results in the production of about one-half equivalent of titrable acidity for each gram-atom of carbon transformed in the region from pH 6 to more alkaline reactions. With the oxidation of acetic acid further to CO_2, especially at pH 5, the medium becomes more alkaline while at pH 12 it becomes more acid.

The transformations with formic acid are especially interesting. At pH 12 to 13 one gram-atom of carbon as formic acid is transformed into H_2CO_3 with the production of about one equivalent of titrable acidity; at pH 8 to 9, with little or no acidity or alkalinity; and at pH 5, with the production of nearly one equivalent (0.9) of titrable alkalinity.

Summary

1. Relying upon the law of conservation of matter and from simple reactions in the main, it is shown that transformations from one form of nitrogen to another within the group N_2, urea, NH_4NO_3, NH_4NO_2, and proteins cause very little or no change in titrable acidity or alkalinity. Transformations from any one or all of this group to ammonia cause the appearance of about one equivalent of titrable alkalinity for each gram-atom of nitrogen changed. Transformations from this same group to nitric acid or nitrates result in the production of about one equivalent of titrable acidity for each gram-atom of nitrogen changed.

2. When weak acids and bases are formed, the pH of the medium is important in determining the amount of titrable acidity and alkalinity produced. This is especially true in some of the transformations that may take place in the sulphur and carbon cycles. Charts showing these relationships are given.

The writer is indebted to Dr. C. S. Bisson for many helpful suggestions during the preparation of the manuscript.

University of California
Davis, Calif.

LITERATURE CITED

1. Brioux, C. L. Influence de l'uree employée comme engrais sur le reaction du sol. Compt. Rend. Acad. Sci. (Paris) **179**: 915–917. 1924.

2. Burri, R., and Stutzer, A. Ueber Nitrat zerstörende Bakterien und den durch dieselben bedingten Stickstoffverlust. Centralbl. Bakt. **1**: 392–398; 422–432. 1895.
3. Chibnall, A. C. Leaf cytoplasmic proteins. Jour. Amer. Chem. Soc. **48**: 728–732. 1926.
4. Csonka, F. A., and Jones, D. B. Studies on glutelins. I. Jour. Biol. Chem. **73**: 321–329. II. *Ibid.* **74**: 427–431; III. *Ibid.* **75**: 189–194. 1927; IV. *Ibid.* **78**: 289–292. 1928.
5. ————————, Murphy, J. C., and Jones, D. B. The iso-electric point of various proteins. Jour. Amer. Chem. Soc. **48**: 763–768. 1926.
6. Hoffman, W. F., and Gortner, R. A. Physico-chemical studies on proteins. 1925. I. The prolamines. Colloid Symp. Monograph II: 209–368. New York: Chemical Catalogue Co. 1924.
7. Hodgman, C. D., and Lange, N. A. Handbook of chemistry and physics. 14th ed. Cleveland: Chem. Rubber Publ. Co. 1929.
8. Loeb, Jaques. Proteins and the theory of colloidal behavior. New York: McGraw-Hill. 1922.
9. Mattson, Sante. The laws of soil colloidal behavior. VI. Amphoteric behavior. Soil Sci. **32**: 343–365. 1931.
10. Pearsall, W. H., and Ewing, Jas. The iso-electric point of some plant proteins. Biochem. Jour. **18**: 329–339. 1924.
11. Riegel, E. M. Industrial chemistry, an introduction. New York: Chemical Catalogue Co. 1928.
12. Sacharowa, T. M. Die Abhängigkeit der Denitrifikationgeschwindigkeit von der Reaktion des Mediums. Centralbl. Bakt. **65**: 15–35. 1925.
13. Tague, E. L. The iso-electric points of gliadin and glutenin. Jour. Amer. Chem. Soc. **47**: 418–422. 1925.
14. Waksman, S. A. Principles of soil microbiology. Baltimore: Williams and Wilkins. 1927.

PHOTODYNAMICALLY INDUCED TROPISMS IN PLANT ROOTS[1]

H. F. Blum and K. G. Scott

(WITH FOUR FIGURES)

Metzner (7) found that a positive phototropic response could be induced in the roots of wheat seedlings by growing them in dilute solutions of fluorescein dyes. Normally the direction of growth in these roots is not influenced by light, but the presence of a very small concentration of dye (*e.g.*, 1/500,000) brings about a definite bending of the roots toward the light. This phenomenon is of interest from two standpoints: the information it may give as to the mechanism of tropic bending in plants, and the bearing it may have on the phenomenon of photodynamic action in general. In conducting the investigations described in this paper, we have been concerned with both of these questions.

Method

In most of the experiments the wheat seedlings were grown on cheese-cloth suspended at the surface of a solution contained in a glass vessel. Roots so grown readily penetrate the cheese-cloth mesh and the direction of their growth may be observed through the walls of the vessel. In most experiments a solution was used having the following composition: KH_2PO_4, 0.001 M; KNO_3, 0.05 M; $MgSO_4$, 0.002 M; $CaNO_3$, 0.05 M. This solution has a hydrogen-ion concentration corresponding to about pH 4.5. In some experiments the concentration was adjusted to other values by the addition of 0.1 N KOH. To these solutions, erythrosin[2] was added when it was desired to produce phototropic responses.

It was found that the effect of cylindrically walled vessels upon the direction of the light rays did not interfere with the production of phototropism, and various sized vessels of this type were used to suit the convenience of particular experiments. Light sources were several types of clear tungsten-filament lamps. Various factors modify the magnitude of the reaction, of course, such as the concentration of the dye and the thickness of the dye solution between the light source and the roots; in any one set of experiments these factors were maintained as nearly constant as possible. A convenient arrangement for observing the phenomenon was found to be as follows: The roots were suspended at approximately the center of a cylindrical glass vessel 6 cm. in diameter, in a solution of 1/500,000 erythrosin

[1] This research was assisted by a grant from research funds of the University of California.

[2] Erythrosin Schultz no. 912, made by National Aniline Company. This is tetra-iodo-fluorescein.

at about pH 4.5, with a 40-watt concentrated tungsten-filament lamp, 40 cm. distant from the roots. These were approximately the conditions in most of our experiments; ordinarily they were performed in a black-walled room; otherwise precautions were taken to avoid any appreciable amount of light from sources other than the direct radiation from the experimental source.

FIG. 1. Normal non-phototropic wheat roots, age 10 days. Light source on the right.

Description of the phenomenon

Under the conditions just described the roots show a definite orientation toward the light source within the course of a few hours. Usually the orientation appears as an abrupt bending toward the source of light, regardless of the position of the source (compare figure 1[3] with figures 2[3] and 3[3]). In

[3] It was found necessary to transfer the plants to flat-walled vessels for photographing. This disturbed the roots to some extent so that the orientation appears somewhat less exact than in the original vessels.

some cases, by placing the source above the seedlings, it was possible to produce bending of the roots through almost 180°, so that they grew nearly vertically upward instead of in the normal positively geotropic manner. In some cases a curious unoriented bending occurs before orientation toward the light becomes definite (figure 2); this is probably due to the effect of the dark reaction of the dye, which will be discussed in a later section, and possibly also to the shading of some roots by others at certain points in

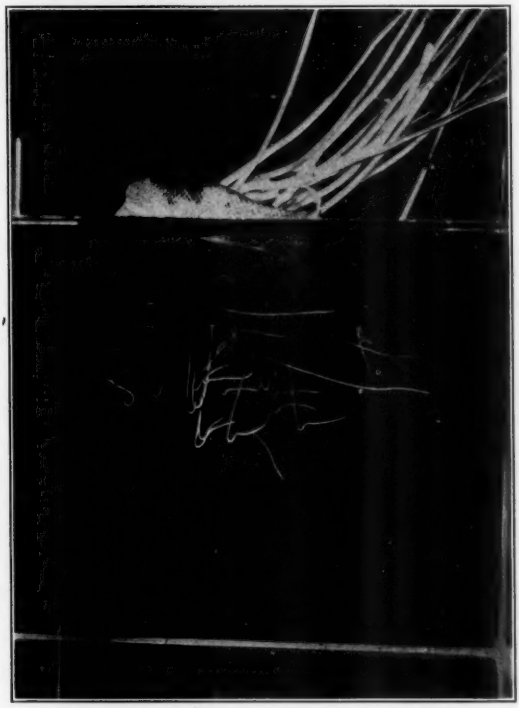

FIG. 2. Wheat roots 10 days old, sensitized after 5 days. Light source on the right.

their bending. The growth of the roots after they have become oriented is usually not in a straight line, but in a series of short waves as is shown in figure 3 at *A*. It would seem that the root is caused to bend toward the light but bends too far and is reoriented.

METZNER (7) has described variations in appearance of the induced tropism. He has separated the phenomenon into two types on this basis, active bending and passive bending. The former, in which the curvature is gradual, is produced by weak light and low dye concentrations. The

latter, in which the curvature takes place at an acute angle, is produced by stronger light and greater concentrations of the dye. We have been able to produce a great variety of shapes by altering the dye concentration and light intensity, but it seems that METZNER'S types simply represent different degrees of the same process, and that there is no real justification for the separation of the two types.

Importance of the growing tip

It was found that the orientation occurs only in the region of the root tip. If a few millimeters of the tip are cut off, the root no longer orients toward a light source. Likewise, if a beam of light is directed above the region of the tip of a normally downward growing root, no bending occurs. This experiment was performed by restricting the light to a narrow band striking the root well above the tip. No bending of roots so illuminated was observed, but roots developing later in the same culture were definitely oriented as soon as they grew down into the beam of light.

These results offer an explanation for the zigzag growth of the oriented roots described above (figure 3 *A*). The extreme tip of the root, or root cap, is composed of a large number of cells which proliferate from the root meristem but do not contribute to the elongation of the root; the significant length growth takes place in the region just behind the root cap, as a result of active cell division and increase in cell size in a limited zone. The phototropic orientation is apparently accomplished by a differential growth on opposite sides of this zone of active growth, the growth being less rapid on the side toward the light source, so that the root tip bends in that direction. In paraffin sections this differential growth appears as a less degree of elongation of the cells on the illuminated side of the root than on the dark side. When by this process of differential growth the long axis of the root becomes oriented parallel to the light rays, the apical region of the root shields the actual growing region from the light, so that the effect of the light upon growth ceases. The differential rate of growth continues for a time, however, until the tip reaches a position at which it is differentially illuminated again. The side of the root previously least illuminated now receives the greater amount of light and orientation occurs in the opposite direction. This process is repeated, producing the effect illustrated in figure 3 at *A*.

Under the microscope we have been unable to determine from paraffin sections whether there is any degree of penetration of the dye into the roots. The macroscopic staining of the roots, however, indicates a considerable accumulation of the dye at the surface at least. This staining is unequal, in general being greatest in the root cap and least in the region of growth just behind. It is probable that the least viable cells are the most readily stained, as is characteristic for fluorescein dyes, and that the staining in the

less rapidly growing regions may be due to the accumulation of the dye in dead or dying cells, particularly in the region of the root cap where cells are being constantly sloughed off under normal conditions. Certainly there is no indication that the macroscopic accumulation of the dye marks the region of greatest effective photochemical action.

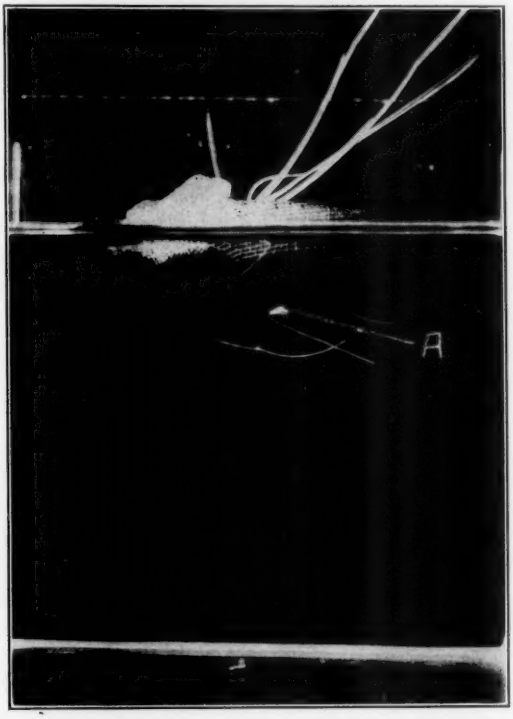

FIG. 3. Wheat roots 10 days old, sensitized after 2 days. Light source on the right.

Dark reaction

Most photodynamic dyes produce destructive effects in cells when in sufficient concentration, even when they are carefully protected from light (BLUM **3**). This is particularly true of the fluorescein dyes, so that it is not surprising to find a marked effect of non-irradiated erythrosin on wheat roots. BOAS and MERCKENSCHLAGER (**4**) found that the roots of sprouting barley seeds were caused to undergo strange, unoriented bending by the addition of fluorescein dyes to the solution in which they were sprouted, although they were not actually growing in the solution. Our wheat roots

were likewise found to exhibit unoriented bendings within a few hours after they were introduced into the erythrosin solutions, although carefully maintained in the dark; these would seem to be similar to those described by BOAS and MERCKENSCHLAGER. It was also observed that growth of the roots was distinctly delayed. It seems probable that the bending is due to the unequal rate of penetration of the dye on opposite sides of the root, growth being delayed most on the side where penetration is the most rapid.

BLUM (1) suggested that the photodynamic phenomena as observed are the resultant of two reactions, the dark reaction and the photo-reaction. BLUM and MCBRIDE (2) have also shown that the two reactions are essentially different in that the photo-reaction requires the presence of molecular oxygen, whereas the dark reaction does not. It would seem that in the case of plant roots the dark reaction is capable of producing root bending by the differential delay of root growth, but that this differential effect is increased and given a definite orientation by the light reaction. The roots usually continue in a more nearly straight-line growth after the first bending, when it may be assumed the dye has penetrated more evenly at all surfaces of the root.

Parallel with the findings of JODLBAUER and HAFFNER (6) and of BLUM (1) that the hemolysis of red blood cells by non-irradiated fluorescein dyes is favored by increased hydrogen-ion concentration, it was found, in general, that the bending of the roots was more pronounced at pH 4.6 than at pH 7, and that the delay of root growth was also greater in the more acid solution. If the hypothesis of BLUM (1) is correct, the phototropic orientation involves two important components, the photo-reaction and the dark reaction. The first, of course, varies with the concentration of dye and with the intensity of incident light; the second should vary with the concentration of dye and with the hydrogen-ion concentration. Optimum conditions for phototropic bending must depend upon the proper selection of conditions with respect to these factors, and also, apparently, with the age and normal rate of growth of the root. It is difficult, therefore, to make generalizations with regard to the rôle of the various factors involved. For instance, our observations indicate in general that the phototropic bending is more pronounced at pH 4.6 than at pH 7.0. With strong light intensities this may be reversed, however, owing apparently to the delay of growth in the more acid solution to such an extent that the phototropic curvature is not observable.

Active radiation

Numerous investigators have made approximate determinations of the wave lengths of radiation active in bringing about photodynamic effects (BLUM 3). All such determinations indicate that these effects are depen-

dent upon a photochemical reaction in which the dye molecule is activated by light. The following experiments have been conducted to determine the wave lengths active in inducing phototropic bending in wheat roots sensitized with erythrosin.

In these experiments a 500-watt Mazda lamp of the projection type was employed as a source, and various Corning glass filters were used to restrict the wave lengths incident on the photosensitized roots. The energy incident upon the roots was varied by placing them at different distances from the source, the reciprocal of the square of the maximum distance at which orientation of the roots in the direction of the source could be determined being taken as an index of the threshold intensity.

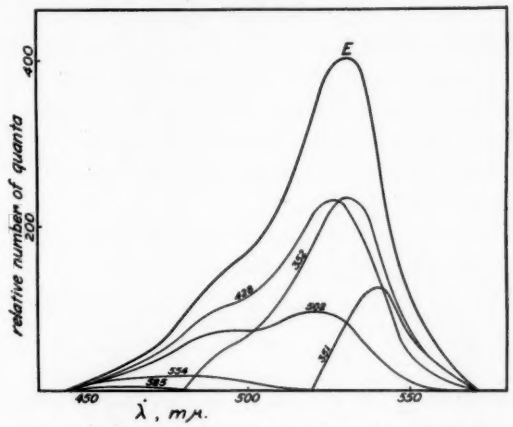

FIG. 4. Absorption curves of dye (E) and filters used.

It was found that no orientation could be obtained when the light was passed through Corning filter 348, which absorbs all radiation in the visible below 570 mμ, or through Corning filter 986, which absorbs between approximately 400 and 700 mμ. Thus the wave lengths active in producing orientation lie between 400 and 567 mμ. The erythrosin solution which was used in the experiments absorbs in the visible between 445 and 570 mμ, so that these experiments indicate at once that the active wave lengths lie within the absorption spectrum of the dye.

A still more definite correspondence between the active radiation and the absorption of the dye is shown by an analysis of the data for filters transmitting in the region of absorption of the dye. It is obvious that the true threshold intensity should be that quantity of incident radiant energy which is absorbed by the photosensitizer in the roots. This quantity of energy was estimated in the following way. The transmittance of the dye

solution used (1/500,000 erythrosin) was determined for various wave lengths by means of a spectrophotometer. According to BEER's law for absorption in solutions

$$-\log T_\lambda = lc\,K_\lambda \qquad (1)$$

where T_λ is the transmittance or fraction of the incident light of wave length λ which is transmitted, l the thickness and c the concentration of the absorbing solution, and K_λ a constant for the particular wave length λ. Assuming that the sensitized region in the plant root represents a given thickness of dye solution of a given concentration, l and c may be taken as constant, so that

$$-\log T_\lambda = K'_\lambda \qquad (2)$$

Thus $-\log T_\lambda$ may be taken as an index of the relative absorption of light of wave length λ by the dye in the sensitized region of the root, regardless of the actual concentration of dye or the thickness of this region. The values of $-\log T_\lambda$ must be corrected for the emission of the source, and when filters are used, for the transmission of the filter. If L represents the corrected values, we may write

$$L = I_\lambda S_\lambda (-\log T_\lambda) \qquad (3)$$

where I_λ is the energy emitted by the source and S_λ the transmittance of the filter at this wave length. When no filter is used S_λ is unity, of course; and when a filter is used whose transmission is outside the absorption of the dye, S_λ is 0.

Relative values of I_λ for various wave lengths were obtained by calculating from WIEN's equation (HARRISON 5) the emission of a black body at 3280° K. This latter value is the maximum color temperature for a 500-watt Mazda projection lamp, the type used as a source. The values of S_λ for the various filters used were determined by the spectrophotometer.

Further corrections are necessary for a rigid treatment which, however, do not greatly alter the magnitude of the threshold values obtained. Actually we are not interested in the incident energy but in the relative number of light quanta incident.

If N_λ is the number of quanta for intensity I_λ

$$N_\lambda = \frac{I_\lambda}{h\nu} \qquad (4)$$

and since $\nu = \frac{c}{\lambda}$

$$N'_\lambda = \frac{I_\lambda}{h\frac{c}{\lambda}} \qquad (5)$$

where ν is the frequency, c the velocity of light, and h is PLANCK's constant.

Since h and c are constant, we may write

$$N'_\lambda = \lambda I_\lambda \tag{6}$$

where N_λ is the relative number of quanta.

Correcting according to (3) we obtain

$$N'_\lambda = \lambda I_\lambda S_\lambda (- \log T_\lambda) \tag{7}$$

Under the conditions of our experiment the light must traverse a given thickness of dye solution before reaching the root. The absorption in this layer is not, of course, the same for the different wave lengths. In our experiments the thickness of solution between the root and the source was not exactly the same in all cases, but may be assumed as 1 cm. since the roots were suspended as near the center of tubes 2 cm. in diameter as was reasonably possible. To correct for this factor the values of N_λ were all multiplied by the transmittance of 1 cm. of the dye solution. Since T_λ was calculated for 1 cm. of solution, the equation corrected for this factor becomes

$$N'_\lambda = \lambda I_\lambda S_\lambda T_\lambda \tag{8}$$

This latter correction may amount to 30 per cent. of the light quanta at certain wave lengths.

In figure 4, *E* represents the absorption curve of the dye corrected as above, and curves for the various Corning glass filters used are each indicated by the number of the filter. The areas under the various curves were obtained by measuring with a planimeter. The ratio of the area under any curve to the area under the total absorption curve *E* indicates the proportion of the light absorbed after passing a given filter to the total absorption without a filter. These ratios are the values A in table I. The relative value of the radiant energy which will just produce orientation of the sensitized root, *i.e.*, the threshold value, should be

$$\frac{A}{d^2}$$

where d is the distance from the source at which orientation can just be detected.

Obviously, if our assumptions are correct, there should be a certain degree of agreement between these threshold values. This is indicated by the data in table I, where the values of $\frac{A}{d^2}$ are certainly of the same order. Some difficulty was incurred in selecting the distance at which orientation first occurs. Since the roots are caused to change their direction of growth by the action of the dye even in the absence of light, some roots will nearly

TABLE I

(See text for explanation of symbols)

Filter	A	d_σ (meters)	$\frac{A}{d^2} \times 10^2$	Tropism
0	1.00	8.00	1.6	+
		8.00	1.6	0
351	0.16	3.32	1.4	+
		4.00	1.0	0
554	0.045	1.52	2.0	+
		1.50	2.0	0
352	0.48	6.17	1.3	+
		6.17	1.3	0
585	0.007	0.53	2.5	+
		0.60	1.9	0
502	0.30	3.62	2.3	+
		4.00	1.9	0
428	0.60	6.20	1.6	+
		6.50	1.4	0
348	0.00	1.10		0
246	0.00	0.10		0
986	0.00	0.25		0
			Average = 1.8	

always be directed toward the source. For this reason orientation was considered to have taken place only when a majority of the roots showed a characteristic sharp bending toward the light. To indicate the good agreement of the values of d thus taken, the values have been recorded in table I for both the greatest distance at which orientation was observed and the least distance at which no orientation occurred; these values were obtained from the results of five to eight experiments. It will be seen that the deviation from the average value is within 40 per cent., which may be considered as good agreement for this type of experiment. More exact values could probably be obtained by more precise measurements, but those obtained show a sufficiently close agreement to indicate definitely that the primary reaction in the phototropic phenomenon is the activation of the dye molecule by light.

Question of oxygen requirement

Attempts were made to determine whether the removal of oxygen would inhibit the phototropism, since, as pointed out by Blum (3), molecular oxygen is characteristically required in photodynamic phenomena. It was found, however, that removal of the oxygen to a low level by bubbling N_2 through the solution inhibited the root growth in itself, so that fair comparison could not be made with roots grown in air. Although this question

remains without definite answer for the present, it may be fairly assumed that molecular oxygen is necessary for induced tropisms, since this has been demonstrated in numerous other photodynamic phenomena.

Discussion

The phenomenon of photodynamically induced tropic bending in wheat roots would appear to be based upon the same type of photochemical reaction as other photodynamic effects, namely, an oxidation of cell constituents by molecular oxygen, the dye acting as a photosensitizer. The effects of this reaction are modified, apparently, by the dark reaction of the dye. The bending appears due to a differential growth on the two sides of the root, whatever the mechanism of its production. The dark reaction may play a very important part in this differential growth, since in general the total length of roots grown in the dark in the dye solutions is found to be considerably less than those grown in the absence of dye. The introduction of dye causes unoriented bending of the roots, probably due to unequal penetration and consequent unequal inhibition of growth on opposite sides of the root as suggested above. It may be that the photochemical reaction itself serves only to alter the permeability of the growing cells to the dye, thus giving an orientation to the delay of growth by the dye. This question, of course, cannot be answered by our experiments.

The bearing of the phenomenon on tropisms in non-sensitized plants is difficult to determine at present. It seems reasonable to propose, however, that phototropic bendings may be based upon photochemical reactions of the same type as the photodynamic phenomena. In such case it would be necessary to assume that a photosensitizing substance of the general type of the photodynamic dyes is present. This is not an unreasonable assumption, since various porphyrins are found among plants and such compounds generally produce photodynamic effects. Further generalization cannot be made without thorough cytological investigations.

Summary

1. Phototropic bending in the roots of wheat seedlings induced by the action of erythrosin and light are described.

2. It is probable that the phototropic bending represents the combined effect of the photo-reaction and the dark reaction of the dye.

3. The wave lengths of light producing the phototropic bending correspond to the absorption spectrum of the dye, indicating that the dye acts as a photosensitizer.

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. Blum, H. F. Studies of photodynamic action. II. The relationship between hemolysis by irradiated and non-irradiated eosine. Biol. Bull. **59**: 81–94. 1930.
2. ————, and McBride, G. C. Studies of photodynamic action. III. The difference in mechanism between photodynamic hemolysis and hemolysis by non-irradiated eosine. Biol. Bull. **61**: 316–323. 1932.
3. ————. Photodynamic action. Physiol. Rev. **12**: 23–55. 1932.
4. Boas, F., and Merkenschlager, F. Reizverlust, hervorgerufen durch Eosin. Ber. deutsch. bot. Ges. **43**: 381–390. 1925.
5. Harrison, G. R. Instruments and methods used for measuring spectral light intensities by photography. Jour. Optical Soc. Amer. **19**: 267–316. 1929.
6. Jodlbauer, A., and Haffner, F. Über die Wirkung von Eosin und Rose bengale auf rote Blutkörperchen und den Zussammenhang von Aufnahme und biologischer Wirkung. Pflüger's Arch. ges. Physiol. **189**: 243–260. 1921.
7. Metzner, P. Über induzierten Phototropismus. Ber. deutsch. bot. Ges. **41**: 268–274. 1923.

NUTRIENT ELEMENTS USED BY LEAVES AND GROWTH OF APPLE TREES[1]

EDMUND BURKE AND H. E. MORRIS

Introduction

Considerable study has been given to the influence of fertilizers on the chemical composition of various parts of the tree and of the fruit, but the problem as to when the tree requires its greatest food supply has received comparatively little attention. Previous work by the writers indicates that the blossoms, leaves, and early growth remove much of the plant foods stored in the tree during its dormant period and that during the early growth the tree makes its greatest demand on the nutrient elements in the soil. The purpose of the present study was to determine to what extent the new leaves and early spring growth utilize the plant foods stored in the dormant tree, and the amount taken from the soil. The samples of apple tissue used in this investigation were obtained from the McIntosh-Morello Orchard in the Bitter Root Valley, Montana.

MURNEEK (**4**) maintains the necessity of an abundance of nutrients at certain critical periods for apple trees, such as during full bloom and preceding the June drop. ROBERTS (**5**) suggests that the failure to set is due to nutritional causes rather than to a lack of pollination or fertilization. GOURLEY (**1**) states that twig growth practically ceases by July 1, and shows by charts that the greatest rate of growth is during the early growing period. HOWLETT (**2**) gives in tabulated form the amount of nitrogen removed by flowers up to full bloom, and in his discussion of the relation of water supply to first drop states: "However, the question is still undecided as to whether the fall of bloom from spurs under ordinary circumstances is not due more to a deficiency of nitrogen than to a deficiency of water." LOEW (**3**), after analyzing the bark of cherry trees for several seasons before and after full bloom, found that after the blooming period the bark had lost 37.16 per cent. of protein, 30.35 per cent. of fat, and 40.59 per cent. of starch.

No attempt is made to cite all the literature relating to the importance of an abundance of plant nutrients when the tree is in blossom and leafing out. Suffice it to say that the value of an abundance of plant foods in the tree and in the soil has been recognized by several investigators.

Unpublished data by the writers show that fertilizers, especially nitrogenous fertilizers, do have a decided influence on the chemical composition

[1] Contribution from Montana State College, Agricultural Experiment Station, Paper no. 28, Journal Series.

of the apple fruit, twigs, leaves, and fruit spurs; and that the blossoms, young leaves, and new wood growth are exceptionally rich in nitrogen, phosphorus, and potassium. In the blossoms, young leaves, and new wood the percentages of these elements were greater than at any subsequent period. As a matter of fact, there was a decrease in the percentages of these plant nutrients in the leaves from their early formation until they dropped in the autumn. The twigs contained the greatest percentages of nitrogen and phosphorus while the tree was dormant, and the least at about the time the leaves had attained their normal size. These results seem to indicate that the greatest demand on the plant nutrients in the tree and soil is made at about the time the tree is in full bloom and the leaves and early growth are very active.

Experimentation

In the spring of 1930, two apple trees of nearly equal size and vigor, growing near each other and about 20 years old, were selected for analysis. These trees were growing on a soil low in plant food, especially nitrogen, hence the annual terminal growth had not averaged more than 3 to 4 inches. One tree, designated the dormant tree, was removed April 9, while in a dormant state and the other, designated the active tree, was removed June 11, at about the time the leaves had attained their normal size.

The material was divided into root, trunk, large limbs, small limbs, and twigs. The green weight of each division, except the roots, was determined before samples were taken for analysis. Samples of the 1926, 1927, 1928, and 1929 growth were also taken for analysis. In addition the leaves and spring (1930) growth were removed from the limbs of the active tree, weighed, and sampled.

The nitrogen, phosphorus, calcium, and magnesium contents were determined by methods adopted by the Association of Official Agricultural Chemists. The potassium content was determined by the perchloric acid method. The moisture content of each sample was determined. A sufficient quantity of roots from each tree was dug to secure representative samples, but no attempt was made to remove the entire root system. The results of all analyses are calculated on the water-free basis.

Chemical data

Table I gives the percentages of five mineral nutrients found in the trees. It shows that in the dormant tree there is a gradual reduction in the percentages of these plant nutrients from the 1929 growth to the trunk, but that the roots are relatively rich in these nutrients. This general trend is also apparent in the rapidly growing tree. It is of interest to note that the percentages of all plant nutrients, except potassium, are higher in the

dormant tree. This indicates that a considerable amount of these nutrient elements is stored in the tree and its root system during the dormant period. Potassium is not stored in the newer wood, but is found in largest quantities in the large limbs, trunk, and roots of the dormant tree.

TABLE I

PERCENTAGE OF NITROGEN, PHOSPHORUS, POTASSIUM, CALCIUM, AND MAGNESIUM IN A DORMANT AND IN A RAPIDLY GROWING APPLE TREE

(PERCENTAGES CALCULATED ON WATER-FREE BASIS)

MATERIAL	NITROGEN	PHOSPHORUS	POTASSIUM	CALCIUM	MAGNESIUM
	TREE NO. 1 (DORMANT)				
	%	%	%	%	%
1929 growth	0.900	0.1787	0.484	1.948	0.1455
1928 growth	0.565	0.1156	0.191	0.985	0.0860
1927 growth	0.405	0.1068	0.177	0.953	0.0740
1926 growth	0.380	0.0855	0.164	0.904	0.0724
Small limbs	0.245	0.0655	0.135	0.629	0.0560
Large limbs (scaffold)	0.195	0.0409	0.147	0.534	0.0411
Trunk	0.120	0.0293	0.098	0.285	0.0355
Roots	0.450	0.1579	0.394	0.655	0.0842
	TREE NO. 2 (ACTIVE)				
Leaves and 1930 growth	1.800	0.3720	1.840	1.419	0.2741
1929 growth	0.425	0.1263	0.490	1.583	0.0678
1928 growth	0.335	0.1008	0.351	1.124	0.0508
1927 growth	0.250	0.0957	0.299	0.955	0.0531
1926 growth	0.250	0.0831	0.245	0.975	0.0557
Small limbs	0.195	0.0813	0.219	0.883	0.0548
Large limbs (scaffold)	0.130	0.0534	0.059	0.471	0.0415
Trunk	0.095	0.0464	0.043	0.219	0.0183
Roots	0.175	0.1212	0.039	0.537	0.0688

The storage of potassium during dormancy differed from that of other nutrients examined, in that the percentages were considerably lower in the 1926 to 1929 growth, inclusive, in the dormant tree than in the rapidly growing tree. The analyses indicate that the percentages of potassium present in the different parts of the tree probably are influenced largely by sap flow. As the sap recedes most of the potassium finds its way to the roots

and lower portions of the tree, where it remains until the sap again rises. Insoluble compounds of potassium are rare and it is reasonable to assume that most of the potassium is in solution and that its distribution is governed by the flow of the sap.

Nitrogen and phosphorus differ from potassium in that they form many insoluble organic compounds. It is therefore reasonable to suppose that during the autumn months they migrate from the leaves to the new wood where they form insoluble compounds and remain during the dormant period. A portion of these elements, however, does find its way to the older wood and even to the roots.

Calcium behaves somewhat like potassium. With the exception of the 1929 growth, the percentages of calcium are higher in the upper portion of the active tree than in the dormant tree, which indicates an upward movement of the calcium in the tree when it became active.

Magnesium behaves more nearly like nitrogen and phosphorus in its distribution during the flow of sap, and it probably forms insoluble compounds which are stored in the younger growth during the dormant period.

Assuming that the two trees are alike, it is apparent from the data presented that the leaves, blossoms, and new growth make a tremendous demand on the plant food stored in a dormant tree.

TABLE II

WEIGHT OF NITROGEN, PHOSPHORUS, POTASSIUM, CALCIUM, AND MAGNESIUM IN DIFFERENT PARTS OF TREES NO. 1 AND NO. 2. TREE 1 (DORMANT) SAMPLED APRIL 9, 1930; TREE 2 (ACTIVE) SAMPLED JUNE 11, 1930

PARTS	DRY WEIGHT	NITROGEN	PHOSPHORUS	POTASSIUM	CALCIUM	MAGNESIUM
	TREE NO. 1 (DORMANT)					
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Twigs and small limbs	17,288	87.99	19.54	39.24	192.93	15.39
Large limbs (scaffold)	11,088	21.62	4.53	16.30	59.21	4.56
Trunk	5,532	6.64	1.60	5.42	15.77	1.94
Total	33,908	116.25	25.67	60.96	267.91	21.89
	TREE NO. 2 (ACTIVE)					
Leaves and 1930 growth	5,625	101.25	20.93	103.50	79.76	15.41
Twigs and small limbs	15,470	45.64	15.01	49.66	169.09	8.66
Large limbs (scaffold)	10,853	14.11	5.75	6.40	51.12	4.56
Trunk	4,703	4.47	2.16	2.02	10.30	0.85
Total	36,651	165.47	43.85	161.58	310.27	29.48

The total weights in grams of the five nutrients in the trees, exclusive of the roots, are given in table II. The active tree was somewhat smaller than the dormant tree, and even if the percentages of plant nutrients had been as great the total weight would be less. The data given in table III are a recalculation of the data given for the active tree in table II, assuming that, exclusive of 1930 growth, it was equal in weight to the dormant tree and that the amount of plant nutrients was increased in the same ratio.

According to the data given in table III, there were 116.25 gm. of nitro-

TABLE III

WEIGHT OF NITROGEN, PHOSPHORUS, POTASSIUM, CALCIUM, AND MAGNESIUM WHEN TREE NO. 1 AND TREE NO. 2 ARE CALCULATED TO EQUAL WEIGHTS

PARTS	DRY WEIGHT	NITROGEN	PHOSPHORUS	POTASSIUM	CALCIUM	MAGNESIUM
	TREE NO. 1 (DORMANT)					
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Twigs and small limbs	17,288	87.99	19.54	39.24	192.93	15.39
Large limbs (scaffold)	11,088	21.62	4.53	16.30	59.21	4.56
Trunk	5,532	6.64	1.60	5.42	15.77	1.94
Total	33,908	116.25	25.67	60.96	267.91	21.89
	TREE NO. 2 (ACTIVE)					
Leaves and 1930 growth	6,147	110.65	22.87	113.10	87.16	16.84
Twigs and small limbs	17,288	60.00	16.77	55.49	188.96	9.68
Large limbs (scaffold)	11,088	14.41	5.88	6.21	52.22	4.66
Trunk	5,532	5.26	2.54	2.38	12.12	1.00
Total in twigs, small limbs, large limbs, and trunk	33,908	79.67	25.19	64.08	253.30	15.34
Grand total	40,055	190.32	48.06	177.18	340.46	32.18

gen in the dormant tree and 79.67 gm. of nitrogen in the active tree, exclusive of leaves and 1930 growth. This means that the leaves and 1930 growth have drawn on the reserve nitrogen supply to the extent of 36.58 gm., but as the leaves and 1930 growth contained 110.65 gm. of nitrogen, the additional supply of 74.07 gm. was derived from the roots and soil.

The total weight of phosphorus in the dormant tree was 25.67 gm., while that in the active tree, exclusive of leaves and 1930 growth, was 25.19 gm., a loss of less than 0.5 gm. The leaves and 1930 growth contained 22.87 gm., however, an excess which must have come from the roots and soil.

The weight of potassium was 60.96 gm. in the dormant tree and 64.08 gm. in the active tree, exclusive of the 1930 growth. The 1930 growth and leaves contained 113.10 gm., making a total of 177.18 gm. of potassium in the active tree. This means that 116.18 gm. had to be taken from the roots and soil. It is evident from the analyses given in table I that a considerable portion of that amount was taken from the roots, for the percentage of potassium in the roots of the dormant tree was ten times the percentage found in the roots of the active tree.

The weight of calcium found in the dormant tree was 267.91 gm., while that found in the active tree, exclusive of leaves and 1930 growth, was 253.30 gm. This means that the active tree, exclusive of leaves and 1930 growth, had lost 14.61 gm. while the leaves and 1930 growth had taken up 87.16 gm. From this calculation the leaves and 1930 growth took 72.55 gm. of calcium from the roots and soil.

The weight of magnesium in the dormant tree was 21.89 gm. against 15.34 gm. in the active tree, exclusive of leaves and 1930 growth. The loss in the active tree was 6.55 gm. This means that the leaves and 1930 growth derived 10.29 gm. of magnesium from the roots and soil.

Discussion

In considering the data presented it must be remembered that they represent the analyses of only one set of trees, and it is doubtful whether the analyses of another set would check these data in every detail; but any differences would probably not affect the interpretations. The percentages of nitrogen, phosphorus, and potassium in the 1929 growth of the dormant tree (table I) were comparable with those found in twigs collected from other orchards in the same fruit section where apple trees were making a greater growth and producing normal crops. However, the nitrogen in the leaves and 1930 growth collected on the same day from adjacent orchards contained from 2.12 to 2.25 per cent. Leaves from trees fertilized with 2 pounds of ammonium sulphate and 2 pounds of sodium nitrate per tree, in the spring of 1929 and in 1930, contained as much as 2.57 per cent. of nitrogen. These data indicate that the amount of nitrogen in the active tree and in the soil was not sufficient for normal growth. The lack of plant nutrients was further indicated by the fact that this tree blossomed heavily but failed to set fruit.

The phosphorus content of the leaves and 1930 growth of the active tree was somewhat greater than the content of comparative samples taken from unfertilized trees in another orchard, and from trees which had received a liberal application of phosphate fertilizer in the springs of 1929 and of 1930. This indicates that the phosphorus supply was sufficient for normal growth and fruiting.

The potassium content in the leaves and 1930 growth of the active tree was somewhat lower (1.84 per cent. as compared with 1.80 to 2.25 per cent.) than that found in ten samples from other trees which were making normal growth and fruiting. The leaves and new growth make a heavy potassium demand on the tree and soil, yet it is doubtful whether this percentage was sufficiently low to affect the fruiting or to interfere with the photosynthesis of the leaves.

Summary

1. Knowledge regarding the nutrient requirements of a tree, especially during early spring, is of great importance in conducting fertilizer experiments.

2. The results of this investigation showed that the leaves and new growth of an apple tree secured from the roots and soil 66.9 per cent. of their nitrogen, 97.9 per cent. of their phosphorus, 100.0 per cent. of their potassium, 83.2 per cent. of their calcium, and 61.1 per cent. of their magnesium.

3. The active tree made a large demand on the nitrogen supply, reducing the amount stored in all parts of the tree.

4. Nitrogen stimulates tree growth more than either phosphorus or potassium, and when it is found in the soil in an available form in sufficient quantities the tree makes a normal growth and the leaves are large and dark green in color. The leaves on the active tree were small and light green in color, which confirms the belief that a lack of nitrogen was responsible for subnormal growth and failure to set fruit.

5. The phosphorus demand decreased the supply in the 1926 to 1929 growth and in the roots, while the supply in the large and small limbs increased. Comparative data indicate an ample supply of phosphorus for normal growth.

6. The percentage of potassium increased in all parts of the tree above the small limbs, but showed a decided decrease in the large limbs, trunk, and roots. The supply of potassium in various parts of the tree is largely influenced by the sap flow.

7. Calcium behaved similarly to nitrogen, and magnesium was similar to potassium in its movement.

8. A tree making rapid growth in the spring cannot depend entirely upon the nutrients stored within it, but must secure a portion of its supply from the soil.

9. It is important that soils in which trees are grown contain an ample supply of nutrient elements and that the soil be sufficiently moist to keep them available for early spring growth.

AGRICULTURAL EXPERIMENT STATION
MONTANA STATE COLLEGE
BOZEMAN, MONTANA

LITERATURE CITED

1. GOURLEY, J. H. Studies in fruit bud formation. New Hampshire Agr. Exp. Sta. Tech Bull. 9. 1915.
2. HOWLETT, F. S. The nitrogen and carbohydrate composition of the developing flowers and young fruit of the apple. Cornell University Agr. Exp. Sta. Memoir 99. 1926.
3. LOEW, OSKAR. Zur Theorie der blutenbildenden Stoffe. Flora **94**: 124–128. 1905.
4. MURNEEK, A. E. Nitrogen and carbohydrate distribution in organs of bearing apple spurs. Missouri Agr. Exp. Sta. Research Bull. 119. 1928.
5. ROBERTS, R. H. Apple physiology, growth, composition and fruiting responses in apple trees. Wisconsin Agr. Exp. Sta. Research Bull. 68. 1926.

CONTROLLED THERMOSTATS FOR PHYSIOLOGICAL STUDIES AT LOW, NON-FREEZING TEMPERATURES

F. C. Steward

(WITH TWO FIGURES)

The frequent necessity for temperature control comparable with that commonly practiced in physico-chemical investigations is now somewhat generally recognized by plant physiologists. When merely a single, arbitrarily chosen temperature is adequate, the use of the now familiar gas or electrically heated ovens or thermostats which operate above room temperature offers no difficulty. In some respects, however, the physiological interest in temperatures between 0° C. and room temperature is greater than that in temperatures of the higher ranges. Especially at low temperatures it is unsafe to presume that a physiological process will have a constant temperature coefficient irrespective of the range chosen. For many purposes, therefore, it is desirable to carry out physiological determinations simultaneously at several selected, controlled, low temperatures. Not all laboratories have at their disposal a series of such control rooms, and even where these are available it is not always practicable to adjust them with the precision often required, or to repeat in separate chambers other equipment incidental to the study in question.

The arrangement here described consists of three relatively large water baths which may be maintained continuously at almost any desired temperature greater than 0° C.[1] The design of the baths offers the maximum amount of free space, and their size is adequate for relatively large-scale operations to be conducted in duplicate at each temperature if desired. The total cost is not excessive, and for a moderate outlay provides all the advantages of a large refrigerator cabinet plus three chambers whose temperatures may be accurately controlled at will. The whole arrangement, although compact, is somewhat more extensive than commonly assembled on the laboratory scale.

Cooling unit and cabinet

The recent advances in domestic refrigeration have developed a variety of standard refrigerators which have met with extensive laboratory use. The outlay for the larger finished models, including both unit and cabinet, is usually considerable, and they are not altogether adaptable for experimental purposes. The refrigeration units can be separately purchased at

[1] If necessary, the output of the unit would be adequate to use for at least one bath below 0° C.

much lower cost, however, and incorporated with laboratory apparatus. In the arrangement to be described an Electrolux unit was utilized. This model contains no moving parts, is noiseless in operation, and involves the minimum of installation as it requires only access to the mains and fixed feed and waste pipes for condenser water. The maximum output available from the model used (series 20 unit) is 1200 B. Th. U. per hour (302 Kg. cal.). This is somewhat larger than can be obtained from single units of the domestic type, although there is no reason to suppose that two smaller units operating side by side would not be equally satisfactory. This, however, might involve greater difficulties in mounting.

The unit described normally operates in a vertical position with its cylindrical cooling surface in air, but for the purpose in question it is more suitably immersed in fluid. This can be effected by building around the cooling surface a stout galvanized iron tank ($10 \times 13 \times 26$ inches). To facilitate this arrangement, a rectangular plate may be welded[2] to the horizontal 2-inch pipes (technically known as the "gas heat exchange") connecting with the cooling surface, and to this the tank can be rigidly bolted, all joints being packed with red lead to prevent leakage. In this way the cooling surface can be immersed in any desired fluid, about 150 liters of which are required to cover it. The unit, which weighs some 370 lb. (117 Kg.), is securely bolted to a rigid framework having adequate supports for the cooling tank. It is necessary to reduce radiation loss from the tank to a minimum. To this end, and also to provide much of the convenience of a standard refrigerator, the cooling tank is surrounded by a large, double-walled, insulated cabinet, leaving the boiler, condenser coils, etc., outside at the rear. The space between inner and outer wall (2 in.) is completely filled with sheet refrigerator cork of this thickness. For the walls of the cabinet, horizontally arranged tongued and grooved boards ($\frac{5}{8}$ in. thick) are adequate. In an improvized structure of this kind hinged doors are likely to cause leaks; tightly fitting, "hatch" doors (double-walled and cork-insulated), which can be removed entirely when desired, are much more satisfactory. The cabinet illustrated in the diagram (fig. 1) has one such in the lower front center ($36'' \times 3''$); one in the top ($12'' \times 19''$) allows access to the cooling tank; and a third (in the upper right side) is available for general access to the cabinet and is useful when it is necessary to remove accumulated frost from the sides of the tank. The total internal volume of the cabinet is 16 cu. ft.; of this about 2 cu. ft. are occupied by the cooling tank itself and the remainder (of which about 9 cu. ft. are present as a single unobstructed chamber beneath the cooling tank) is available for general cold storage purposes. Experience has shown that this

[2] This is best done by the manufacturer before the unit is charged with hydrogen and ammonia.

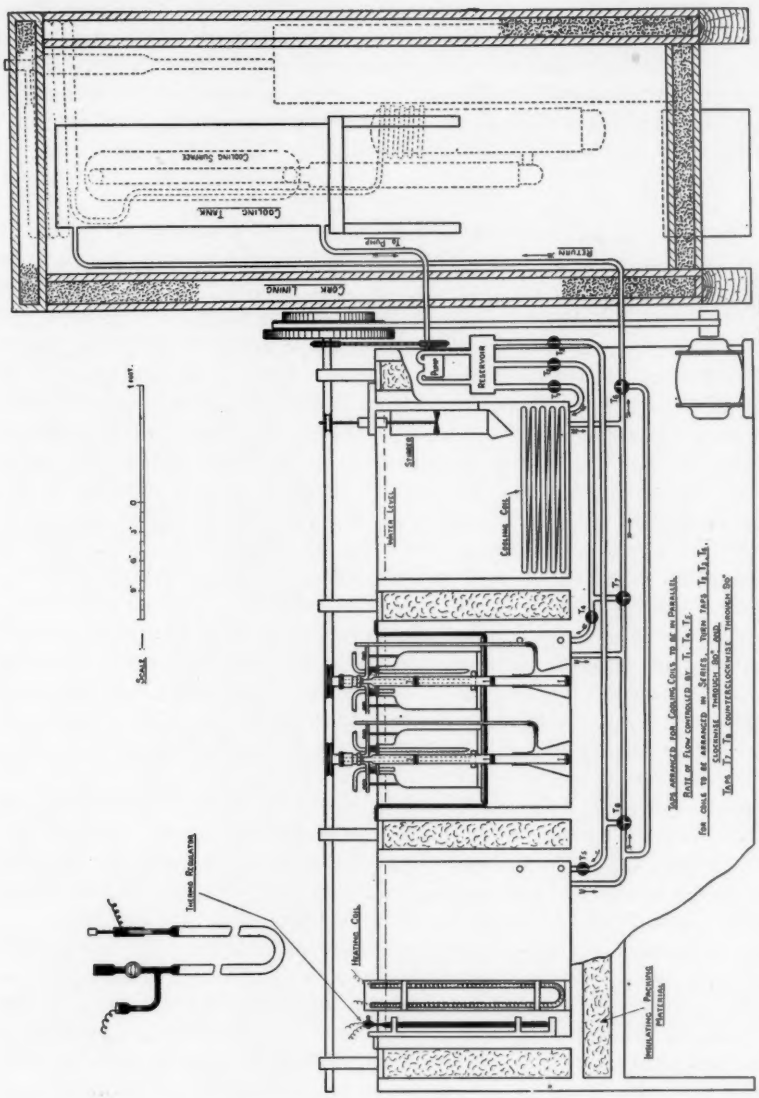

FIG. 1. Diagram of bath and cooling unit. Drawn by N. WARR.

arrangement, constructed by relatively unskilled labor, is effective and has all the convenience of a much more elaborate and expensive cabinet, especially if excessive external demands are not made upon the output of the unit. In the arrangement illustrated in figure 1 no great precision was demanded when using the cabinet, but there is no reason why thermostatic control should not be incorporated if desired. An even larger cabinet could be built around the unit if adequate insulation could be provided.

Circulating system

The cooling surface, immersed in fluid, offers a source of cold which may be distributed at will. Of the two solutions commonly used for refrigeration purposes, calcium chloride and glycerin, the latter is more satisfactory. It appears to be less corrosive to the copper cooling coils, and the viscosity of a 40:60 glycerin-water mixture by volume is not excessive. The maximum efficiency of the unit is obtained at an extraction temperature of $-5°$ C., and the concentration stated is more than adequate to prevent freezing at temperatures below this. The cold glycerin-water mixture, drawn off from the base of the cooling tank, is circulated through copper coils (internal diameter 0.25″, external diameter 0.375″) situated at the base of each of the three thermostats. Figure 1, first compartment, illustrates this point. (To avoid overcrowding the diagram, the features of the three baths are shown in separate compartments in figure 1, although actually the fittings were replicated in all three baths. Also the circulating system has been shown diagrammatically and not exactly as *in situ*.) Each coil has four complete turns and the inlet and outlet are securely fixed to the teak wall with suitable unions. Leads, shown diagrammatically in figure 1, are arranged so that the cooling fluid traverses the three coils separately (coils in parallel) and passes by a common return to the top of the cooling tank. In this way, by controlling the rate of flow (using taps T_1, T_4, T_5) to each of the three coils, the baths can be maintained at different temperatures. Experience shows that much greater control can be exercized and the desired temperatures more readily obtained if the flow through the coils can be changed at will from parallel to series. In the latter case the cooling liquid passes in turn through all three cooling coils at the same rate and three different temperatures are maintained in the baths. For this method the total rate of flow is controlled by tap T_1 (T_2, T_3 are closed) and the two-way taps (T_6, T_7, T_8) are adjusted as indicated in the lower part of figure 1. Since radiation from the external copper leads is considerable, all connections and metal portions must be insulated, leaving only the control taps free. A double layer of tightly wrapped thick felt provides a convenient and effective insulation. To maintain an adequate circulation of the cooling mixture, a small gear pump, such as is used for oil circu-

lation in an automobile engine, is inserted in the cooling circuit. The pump actually in use is the normal fitting for an Austin car, but this has been fitted with a gland around the driving spindle to prevent leakage of the glycerin mixture. The pump is driven by means of a chain and sprockets from the main drive of the apparatus. Two speeds are obtained by using two sizes of sprockets on the pump spindle. Since the efficiency of the cooling system depends upon a uniform rate of flow of the glycerin mixture, the chain drive described is preferable to a belt drive. When using the coils in parallel, the total flow is of the order of a pint a minute, but when using the coils in series it is considerably smaller.

Thermostats

The three thermostats, each of which has a volume of about 5 cu. ft., are constructed of stout teak 1.25″ thick, jointed and the joints sealed with red lead, and have a 3-inch space packed with insulating material surrounding them on two sides and the base (figs. 1, 2). It proved more con-

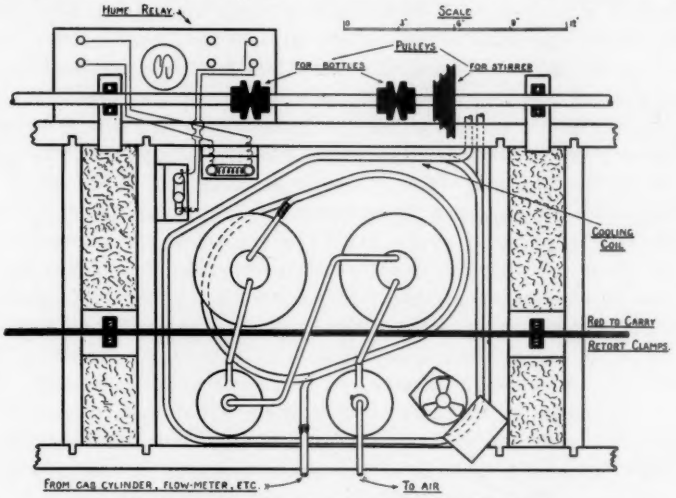

FIG. 2. Plan of one bath. Drawn by N. WARR.

venient to eliminate insulation along the length of the apparatus, as this was required for fixtures (taps, pipes, relay boxes, etc.). The three-vaned stirrers shown pull surface water down a cylindrical chimney, made of sheet zinc, and project it along the base of the bath to the heater and thermoregulator situated diagonally opposite. The stirrer shafts are mounted on ball bearings and operate by endless leather drives from a common shaft ($\frac{5}{8}$″ diameter, mounted on four self-adjusting ball bearings) which revolves

at approximately 200 R.P.M. Each bath is supplied with a heater and thermo-regulator circuit. By slightly overcooling the baths the heaters operate intermittently, and more constant control of temperature is obtained than if the constancy of output of the unit and rate of flow in the coils were the sole regulating mechanism. By this means the temperature variation in the baths does not markedly exceed the sensitivity of the heater and regulator circuit. Thermostatic devices are common and familiar. The one adopted has been described by HUME (1). It has been used with success in other work (2), and is simple and inexpensive, operating directly from A.C. mains. The heaters used were also of the type described by HUME. A long compact U-tube mercury thermo-regulator with one fixed and one adjustable platinum contact was fitted to each bath (fig. 1). It is a simple matter to vary the volume of mercury in the regulator and thus control the temperature at which the relay operates.

Thus far the thermostat fittings, etc., are such that the baths could be adapted to almost any physiological investigation involving various temperatures. With but little alteration the water baths could be converted into air chambers. This actual equipment, however, was intended for use in respiration and salt absorption studies analogous to those described elsewhere (3). A technique for this work under controlled conditions has already been described (2), but this did not include the use of more than one temperature in any one experiment, and this of necessity was greater than room temperature. In fact the large bath there shown has in conjunction with the apparatus here described frequently formed a fourth and higher temperature. Two of the 4-liter containers previously described (2), complete with aeration device, stirrer, and gas-tight mechanical bearings, can be accommodated in each compartment. Figure 1 (second compartment) and figure 2 illustrate the baths complete with two containers for the respiring material, modified Reiset towers for carbon-dioxide determination, and a second copper coil through which the flowing gas for aeration passes before it enters the first of the experimental vessels.[3] The mechanical stirrers for each respiration vessel are also operated from the main shaft by pulleys made in two sections, whose mutual adjustment controls the gear ratio for this drive.

Working directions

It is unnecessary to give detailed working directions. A fairly constant room temperature (15°–18° C.) standardizes the losses due to radiation and increases the stability of the whole system. It is inadvisable to insert the full load suddenly, but more satisfactory to cool down the three compart-

[3] For further details of the devices used in regulating the flow and composition of gas, see (2).

ments one by one to the desired temperature, and then to adjust the flow of the glycerin mixture to the minimum amount necessary to maintain it (with only intermittent use of the heaters), before inserting another bath in the circuit. In this way a low cooling tank temperature is preserved and the unit exerts its maximum output. All possible necessary adjustments (rate and direction of flow of glycerin, stirring, relays, etc.) are under the control of the operator. As approximate criteria of the efficiency of the unit, the temperature of the issuing condenser water, the temperature at the base of the brine tank (which rises when the unit is overloaded), and the presence of ice on the cooling tank and on the external portions of the unit (which indicates a reserve of output) are adequate. When the external circuit is eliminated the three-step control fitted to this unit may be adjusted to maintain a low cabinet temperature (22° F.) without excessive current consumption (500 W).

By using this apparatus it has been possible to carry out efficiently and reliably, over relatively long periods of time, experiments upon respiration and salt absorption at a variety of temperatures between 0° C. and room temperature. It is suggested, however, that the utility of the system described is not confined to experiments of this nature, but that granted ordinary workshop facilities, a complete laboratory unit which embodies a large cold-storage refrigerator cabinet and three accurately controlled low temperature baths can be constructed at a moderate cost.

The writer desires to acknowledge his indebtedness to Professor J. H. PRIESTLEY, who generously provided the facilities incidental to the development of this technique. For some technical mechanical suggestions he is indebted to Mr. S. G. RICHARDSON, of the Department of Engineering, Leeds University, and for technical assistance with regard to the cooling unit to Mr. TAYLOR, of the Leeds branch of the Electrolux Company.

UNIVERSITY OF LEEDS
ENGLAND

LITERATURE CITED

1. HUME, J. A thermostat to work off A.C. mains. Journ. Opt. Soc. of Amer. and Rev. of Scient. Instrum. **19**: 158–161. 1929.
2. STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. II. A technique for the study of respiration and salt absorption in storage tissue under controlled environmental conditions. Protoplasma **15**: 497–516. 1932.
3. ————, WRIGHT, R. W., and BERRY, W. E. The absorption and accumulation of solutes by living plant cells. III. The respiration of cut discs of potato tuber in air and immersed in water, with observations upon surface: volume effects and salt accumulation. Protoplasma **16**: 576–611. 1932.

NEW METHOD OF ARRANGING EXPERIMENTS WITH VEGETABLES IN SAND CULTURES WITH FLOWING SOLUTION[1]

Z. ŻURBICKI

(WITH THREE FIGURES)

There is a field of research in which it is necessary to keep the contents and concentration of nutrient solutions uniform, and therefore sand cultures with flowing solutions are used. The necessary uniformity in the contents of the nutrient solution can be achieved by regulating the flow of the liquid. In the laboratories of PRIANISHNIKOFF (Agrochemical Experimental Station) and in the Agricultural Section this method was successfully used to determine the reaction of plants to different forms of nitrogen nutrition under different conditions, as well as to study the influence of different relations of the elements of the nutrient solution to the development of the plants. By such arrangement of the experiments one can be sure that during the entire period of growth the plants are feeding either with ammonia or with nitrate nitrogen. All possibility of the slightest nitrification is excluded by the rapid flow of the solution. The reaction of the medium varied within narrow limits, fixed by the measurements of pH solution flowing in and out of the vessel.

The method applied up to now, having been taken in principle from that of ALLISON and SHIVE (**1**), is described by DICKOUSSAR (**2**). Part of the arrangement remains unaltered even with the use of mechanization of the flowing sand cultures, therefore it is described briefly. The general view of the apparatus is shown in figure 1. The supply of 16 liters of nutrient solution for two days and sufficient to fill two vessels (B_1 and B_2) is placed in a bottle tightly closed with a rubber stopper. Through the stopper, the glass tube 1 and the siphon 2 are inserted. By means of siphon 2, the solution is transferred into the intermediate flask C. Atmospheric air enters the bottle A through tube 1. The solution will flow through siphon 2 from A to C until the level of the liquid of jar C reaches the height fixed by the position of the lower end of the tube 1, admitting the air. When the liquid at both ends of the siphon comes to a level the transfer of liquid stops. The solution goes from jar C through siphon 3, to vessels B_1 and B_2 along the two branches of siphons 4_1 and 4_2, the speed of flow of the solution being regulated by a screw clamp or glass cock. A uniform speed of flow for the two vessels is fixed approximately, and by experience a speed is selected so that the jar will be drained in a given time. Such regulation is hard to control

[1] Contribution from the Central Scientific Research Institute of the Sugar Industry, Agricultural Section.

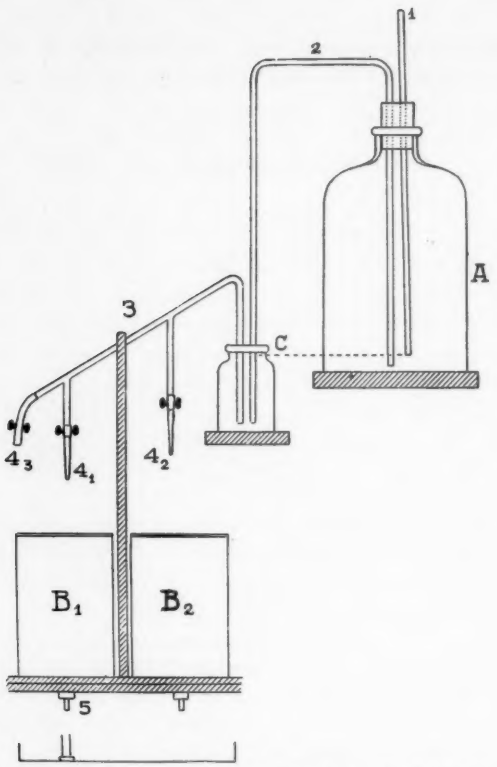

FIG. 1. Diagram of apparatus for control of flowing culture solutions.

and there is no certainty that the two vessels (B_1 and B_2) are getting the same amount of solution, and that the quantity delivered to each will be sufficient for the given time. It is especially difficult to work with the screw clamp since the rigidity of the rubber tube weakens with time, and the flow may stop altogether. The intermediate jar C maintains a steady level and creates a uniform pressure in siphon 3, or it would lower with the diminishing of the liquid in jar A, and the flow of the solution would gradually diminish.

The vessels B_1 and B_2 have at the bottom of each a glass tube closed by a rubber cork, with a glass tube inserted through it. To prevent the sand from entering this glass tube, it is covered with glass wool. (The solution obtained through this glass tube serves to determine the reaction.) The drops of the nutrient solution dripping from tubes 4_1 and 4_2 are falling

always at the same point in the vessel. At the same place there is a column of sand being permeated by the solution. The rest of the area of the vessel is not irrigated by the solution, and the spreading of the solution over the largest part of the vessel is greatly handicapped.

This circumstance is considered to be one of the greatest defects of this system. To start the apparatus it is necessary to force air into the tube 1 until siphon 2 is filled with liquid, then to draw the solution into siphon 3 through tube 4_3, and to regulate the flow through the offshoots 4_1 and 4_2 by means of stop-cocks. During the summer of 1931, the greenhouse of the Agricultural Section modified this method in a manner that greatly facilitated the work. With the new equipment mechanized and uniform distribution of the nutrient solution in the parallel vessels (B_1 and B_2) is obtained. Within 2–3 minutes it can be calculated how long a given amount of solution will last at a given speed of flow; a thorough gravitational flow of liquid through the vessels is also obtained.

The experiment was carried out with four vessels, since work on beets with two vessels is impracticable because of the great variability of crops. All four vessels were supplied with solution from a 16-liter bottle (A) with the same intermediate jar C as in the previous experiment. The siphon 3 was changed. Instead of the two offshoots 4_1 and 4_2 it had only one, which was introduced at the center between the two vessels.

This offshoot is connected with triplet 1 (figure 2). In the upper arm of the triplet there is a glass cock to regulate the flow of the nutrient solution simultaneously to all four vessels. The two side arms of the triplet project 2–3 cm. beyond the border of the adjacent vessel. Through the triplet the nutrient solution is divided into two equal parts for the vessels a and b on one side and for the vessels c and d on the other side. The whole triplet is made of uniform glass tubes (2 mm. in diameter) with side arms drawn out to make the outlets as uniform as possible. The exact division of the flowing liquid into two equal parts is obtained by slight turning of the support 3 in which triplet 1 is clamped. The slightest declination of the triplet from its perpendicular position is sufficient to change the amount of liquid flowing from each arm. The accurate adjustment of the triplet before use may be verified by measuring the flow of the solution. The further distribution of solution between vessels a and b on one side and c and d on the other is accomplished by the swinging curved tubes 2 fastened to a common metal support. The general arrangement of the apparatus can be seen in figure 2. The details of the swinging tubes are given in figure 3. In position 1 (figure 3) the drops of the nutrient solution are falling from the triplet into tube 1; with accumulation of the fluid in the tube the center of gravity of the whole system moves towards the position a, since the solution in tube 1 changes its position to the left of the fulcrum. At a certain

moment tube 1 tips the entire apparatus into position 2; the fluid from tube 1 is flowing into the culture vessel, while the nutrient solution is entering tube 2. After the tube has been filled with a certain amount of solution, it tips the apparatus again into position 1. Tubes 1 and 2 must both be of exactly the same glass and bent at exactly the same angle. Before the experiment is started they are both adjusted to deliver the same amount of nutrient solution. This adjustment is accomplished simply by changing

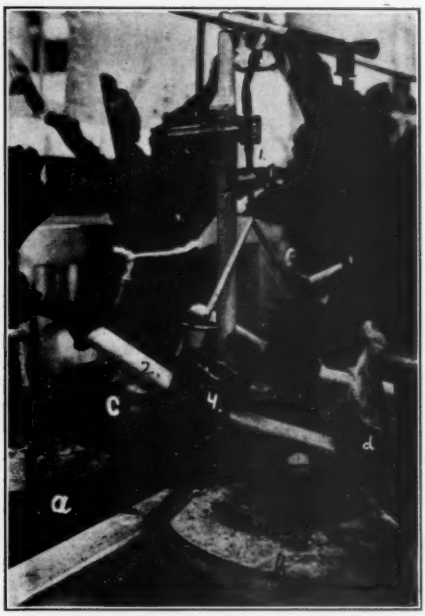

FIG. 2. Photograph of apparatus for regulating flow of culture solutions in sand.

their positions in the holders, and by finally balancing accurately with the additional weight 4 (figure 3), which works along the screw.

Before arranging the experiment the amount of fluid delivered is measured. It is difficult to make the apparatus regulate the flow exactly, but this is not imperative, since it is indifferent for the plants, whether the nutrient solution is supplied in portions of 15 or 17 cc. It is important only that both tubes deliver exactly the same amount of the solution, and distribute it equally between the vessels. The number of cc. the apparatus delivers is recorded for each apparatus.

Every now and then during the vegetative period a check of the setting should be taken. To do this it is not necessary to measure the amount of fluid delivered; it is sufficient merely to check the time necessary to fill one

of the tubes. With exact adjustment both tubes are filled in the same period of time. Since the amount of solution delivered from each tube is given, it is easy to calculate whether the contents of the supply bottle will last until the time of changing the fluid at the set speed of flow. Emptying of the tubes into the vessels is done quickly. To prevent washing out of the sand, several glass plates are introduced in such a way as to have the solution spread over half of the surface of the vessel. Each morning the vessels are turned 180° so that each half may be equally irrigated. By adding to the length and width of the bent collecting tubes, the amount of simultaneously received solution may be increased. This makes possible

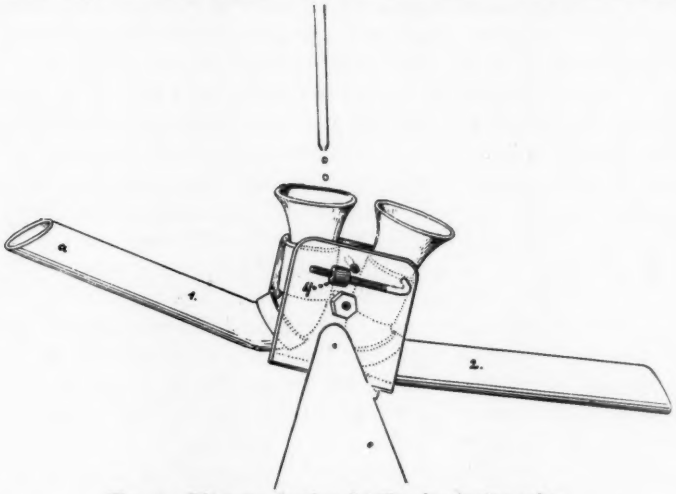

FIG. 3. Diagram showing details of swinging tubes.

a still more uniform irrigation of the entire vessel. The delivery of the solution in portions is greatly to be preferred to drip delivery, which was the old method.

The solution from the siphon is thus distributed into four parts. First the division takes place in the triplet, and second at the bottom ends of it through the swinging tubes. Opening up one cock simultaneously delivers the nutrient solution to four parallel vessels in exactly the same amounts, while before it was necessary to guess at the delivery of the solution to each vessel by regulating a screw clamp. It is not necessary to adjust the cock every day, since the triplet is always full of the solution even if the supply jar is empty. It is sufficient to replace the empty supply jar with a full one, and to add the solution to the intermediate jar to have the entire system at work again.

During the day the temperature of the solution flowing through the

tubes rises noticeably and sometimes causes accumulation of air bubbles in the triplet, which stops the outflow of the liquid. To let the air bubbles escape it is necessary only to give the cock of the triplet a full turn. It is advisable to have an extra set of supply jars, as we did in our experiments, and to prepare the nutrient solution a day before it is needed. This enables one to feed the plants continuously during the growing period.

To prevent the solution from developing algae both jars are covered with black and white paint. All the parts out of the jars are first painted with black lacquer, then with white lead. The white color prevents the solution from getting exceedingly hot. In recently published work, PIRSCHLE (3) recommends covering the surface of the vessels with procelain balls, dipped in a mixture of paraffin with coal. The black color of the balls prevents the vessel from developing algae. PIRSCHLE also rejected the previous methods of sand cultures on account of their defects, which I have pointed out previously, and adopted a system of pouring a certain amount of nutrient solution into the culture vessel by hand every 4 hours.

This method can hardly be called a successful one; besides, it calls for a night shift. It prevents the constant conditions required and introduces changing ones. The humidity in the vessels is changing, since the developing plants in 4 hours are liable during the day time to evaporate a large proportion of the water retained in the sand. The concentration of the nutrient solution is changing, and the reaction (pH) changes very greatly. These changes in reaction might not have been noted in PIRSCHLE's work if the reaction is considered to be the average pH value of the delivered nutrient solution, rather than the pH of the first portions of the solution displaced by the entrance of fresh nutrient solution.

As can be seen from our practice and bibliographical data, the methods applied up to the present do not satisfy investigators. The method suggested here for mechanization of flowing sand cultures is a great step forward in the improvement of the method itself, and also greatly facilitates the care of the experimental cultures.

INSTITUTE OF THE SUGAR INDUSTRY
Moscow, U. S. S. R.

LITERATURE CITED

1. ALLISON, R. V., and SHIVE, J. W. Studies on the relation of aeration and continuous renewal of nutrient solution to the growth of soybeans in artificial culture. Amer. Jour. Bot. **10**: 554–566. 1923.
2. DICKOUSSAR, J. G. Results of vegetation experiments. Agrochem. Exp. Sta. **14**: 287. 1928. Moscow.
3. PIRSCHLE, K. Nitrate und Ammonsalze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionenkonzentration. III. Planta. **14**: 583–676. 1931.

BRIEF PAPERS

CHARACTER OF HEMICELLULOSE IN CERTAIN FRUIT TREES[1]

In extension of earlier observations on the nature of hemicellulose in apple wood,[2] it has seemed desirable to compare the nature of this extractive in different species. Branch tissue of two seasons' growth was taken in August from apple, cherry, and pear; but in the case of peach and plum one year's growth was used. After drying at 55°, with bark attached, the material was ground to pass a 100-mesh sieve. A sample of 100 gm. from tissue freed of lipides, sugars, and starch was hydrolyzed for 3 hours with 2 liters of boiling 1.0 per cent. (wt.) H_2SO_4. Sugars were then separated from the neutralized extract by means of hot alcohol. The loss of weight through extraction was taken as the hemicellulose content and the result-

TABLE I

PERCENTAGE DISTRIBUTION OF FREE SUGARS IN THE HEMICELLULOSE EXTRACT OF FRUIT TREE BRANCHES

SPECIES	HEMICELLULOSE IN DRY WOOD	FREE SUGARS IN HEMICELLULOSE		
		GALACTOSE	GLUCOSE	XYLOSE
Apple	20.1	4.5+	48.2	47.3
Cherry	15.4	0.08*	30.0	70.0
Peach	21.4	0.13*	18.7	81.3
Pear	23.8	3.7+	58.4	37.9
Plum	21.7	2.7+	44.6	52.7

* Values given were obtained by WHITTIER'S procedure (Ind. & Eng. Chem. 16: 744. 1924) following negative values derived by the Official Method; hence the + estimation for the other samples. If, as is highly probable, these values are largely due to presence of galacturonic acid, the values for xylose should be correspondingly reduced.

ing sugars were determined by conventional methods as given in BROWNE'S Handbook of Sugar Analysis. Deduction for the reducing powers of galactose and xylose gave a residual value here expressed as due to glucose, in the assembled data of table I.

The principal variations within the free sugar fraction are seen to be

[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] Jour. Biol. Chem. 45: 407–414. 1921; Ind. & Eng. Chem. 16: 139–192. 1924.

very low contents of either galactose or galacturonic acid in the cherry and peach, together with exceptionally high proportions of xylose in these same species. It was found that the alcohol-insoluble fraction of hemicellulose in both cases contained nearly equal proportions of glucose and xylose. The results for the apple differ somewhat from those reported in the earlier examination cited, but the season of sampling and method of hydrolysis differed also.

Small samples of hemicellulose extract were prepared by the preceding methods. These were neutralized, fractionated, and subjected to determination of uronic acids by the usual decarboxylation procedure. The data obtained are shown in table II. In conjunction with the low yields of

TABLE II

PERCENTAGES OF URONIC ACID EQUIVALENT IN THE HEMICELLULOSE OF FRUIT-TREE WOODS, EXPRESSED ON BASIS OF ORIGINAL DRY WOOD

SPECIES	APPLE	CHERRY	PEACH	PEAR	PLUM
1. Decomposed in hydrolysis	0.21	0.41	0.20	0.20	.....*
2. Free sugar fraction	1.27	0.74	0.90	0.81	1.31
3. Non-free fraction	2.07	1.26	1.54	1.95	0.74
4. Total	3.55	2.41	2.64	2.96	2.05
5. No. 2 as percentage of No. 4	36	31	34	27	64

* Negative value obtained.

mucic acid associated with the cherry and peach, as reflected in galactose values of table I, the values for these species in table II seem to suggest the occurrence of a factor other than galacturonic, such as glucuronic acid. It may be observed that the uronic acid equivalent of the free sugar fraction constituted about 30 per cent. of the total in the hemicellulose in most cases, but twice as much in the plum.

From the use of data in tables I and II, computations have been made of the proportions of both free (*i.e.*, of free sugar fraction) and total uronic acid in the hemicellulose of species here considered. The free acid ranged from about 3 to about 6 per cent., and the total from 10 to 15 per cent., of the hemicellulose. From the point of view of proportions involved, glucose and xylose would seem to be equally significant reserve components in the hemicellulose fraction. It is interesting to note that the supposed conversion of glucose to xylose had been more extensive in these samples of cherry and peach woods than in the other cases. These particular species are notable for their capacity to form pentose gums. Here, also, the transition through the intermediate hexuronic acids has been remarkably complete,

little more than traces of the latter remaining in the tissues.—W. E. TOTTINGHAM, *Department of Agricultural Chemistry, University of Wisconsin.*

EXAMINATION OF STARCH AND HEMICELLULOSE EXTRACTS FROM APPLE WOOD[1]

This brief statement is presented in the hope that it may prove of service to those having occasion to determine starch and hemicellulose in woody tissues. Omitting citation of specific cases, mention may be made of prevalent dissatisfaction with the current method of expressing these fractions in terms of reducing power of their extracts. The observations were made on apple branches of one year's growth, with bark attached. Samples were ground to pass a 100-mesh sieve. After the usual extractions by ether and hot alcohol, the starch fraction was removed by use of salivary amylase. The hemicellulose fraction was extracted subsequently by boiling in 2.0 per cent. H_2SO_4 for one hour.

Based upon the nearly equal reducing power of the common hexoses and pentoses, and the similarity of their anhydride factors, the reducing power of these extracts has been expressed as hexosan. In a preliminary series of samples the percentage of glucosan in the material extracted varied as follows: Sugar fraction 23.5 to 42.7, av. 30.3; starch fraction 41.9 to 88.5, av. 67.6; hemicellulose fraction 16.1 to 35.6, av. 26.9.

In another series of samples the starch and hemicellulose extracts were analyzed for contents of pentosan, uronic acid anhydride, crude protein, and ash, in addition to the usual determination of reducing power. Using the CO_2 yield in distilling with 12 per cent. HCl as a measure of uronic anhydride, it has been assumed[2] that the latter would yield 16.7 per cent. of furfural. KROEBER's tables gave the average factor of 1.7 for converting furfural to its pentosan equivalent. From these two values was derived the factor 0.3 for converting uronic anhydride to its pentosan equivalent. The "true" pentosan content has been derived by subtracting this last equivalent from the total value of the function in question. Subtraction of the pentose equivalent of "true" pentosan from the total reducing power gave a value considered to be the true glucosan or starch. It must be recognized that the uronic acids contribute to this function but in minor degree, as a result of decomposition. There appears to be no basis, however, for evaluating this function.

In eight samples the starch extract gave recoveries as follows: ash 10 to

[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] NANJI, D. R., PATON, F. J., and LING, A. R. Decarboxylation of polysaccharide acids. Jour. Soc. Chem. Ind. (Trans.) 44: 253T–258T. 1925.

TABLE I

STARCH CONTENT OF APPLE WOOD, INCLUDING COMPENSATED VALUES

SAMPLE NUMBER	STARCH EXTRACT*	CRUDE STARCH†	TRUE STARCH‡	URONIC ANHYDRIDE	TRUE PENTOSAN‡	TOTAL COMPENSATED CARBOHYDRATES	CRUDE PROTEIN
	%	%	%	%	%	%	%
1	5.12	2.17	1.79	1.18	0.41	3.38	0.66
2	4.00	2.12	1.44	1.16	0.68	3.28	0.41
3	8.33	4.80	3.50	2.33	1.33	7.16	0.28
4	4.52	2.10	1.22	1.72	0.81	3.75	0.42
5	10.55	5.20	3.80	1.69	1.37	6.86	0.30
6	4.50	2.70	1.89	1.67	0.86	4.32	0.70
7	13.00	8.89	8.06	1.17	0.78	10.01	1.07
8	6.83	3.22	2.60	1.37	0.61	4.58	0.73
Variability of recovery	1.0/4.2	1.0/4.2	1.0/6.6	1.0/2.0	1.0/3.3	1.0/3.1	1.0/3.8

* Loss of weight in extraction.

† As usually determined by total reducing power.

‡ Values as compensated for overlapping determinations.

TABLE II

HEMICELLULOSE CONTENT OF APPLE WOOD, INCLUDING COMPENSATED VALUES

SAMPLE NUMBER	HEMICELLULOSE EXTRACT*	CRUDE HEMICELLULOSE†	TRUE GLUCOSAN‡	URONIC ANHYDRIDE	TRUE PENTOSAN	TOTAL COMPENSATED CARBOHYDRATES	CRUDE PROTEIN
	%	%	%	%	%	%	%
1	29.9	17.0	9.6	5.1	6.0	20.7	1.2
2	27.4	16.4	9.3	7.1	5.5	21.9	0.8
3	31.0	10.3	0.6	7.4	8.4	16.4	0.3
4	31.3	13.6	0.3	6.6	11.9	18.8	0.6
5	30.5	13.5	3.4	7.0	8.5	18.9	0.6
6	28.8	11.3	3.2	7.8	7.2	18.2	0.6
7	24.1	11.5	3.6	3.4	5.8	12.8	1.0
8	32.8	13.4	5.6	3.9	6.2	15.7	1.3
Variability of recovery	1.0/1.4	1.0/1.7	1.0/32.0	1.0/2.3	1.0/2.2	1.0/1.7	1.0/4.3

* Loss of weight in extraction.

† As usually determined by total reducing power.

‡ Value as compensated for overlapping determinations.

26 per cent., av. 15; crude protein 5 to 26, av. 10; uronic anhydride 9 to 37, av. 23; pentosan 6 to 19, av. 12; "true" starch 27 to 62, av. 39; total determinations 89 to 150,[3] av. 100. The individual values have been computed to percentages of their respective samples of wood and the "true" starch compared with the usual expression for this factor. These results appear in table I, wherein successive pairs of samples represent differences in either cultural or developmental conditions which might be expected to entail a lesser starch content in the second sample. It may be seen that the "true" starch content exhibits wider ratios than the conventional starch values, in relation to tissue conditions, while the other carbohydrate constituents vary irregularly.

A similar examination of the hemicellulose extract resulted as follows: ash 9 to 17 per cent., av. 13; crude protein 1 to 4, av. 3; uronic anhydride 12 to 27, av. 21; pentosan 19 to 38, av. 25; "true" glucosan 1 to 34, av. 15; total determinations 65 to 92, av. 77. As computed to percentage of the tissue, the results are presented in table II. From these it appears that no consistent correlation exists between any or all of the carbohydrate constituents and the cultural history of the tissue. Furthermore, the "true" glucosan content varied independently of the "true" starch content. In these respects the hemicellulose fraction seems to be less definitely and directly related to plant performance than is the starch fraction.

It is recognized that the element of assumption is retained in the present treatment. Moreover, the results with one sample indicate that the compensations here applied are not generally applicable without either modification or reservation. Nevertheless the data appear to be more informative than the conventional values. From the quantitative aspect, starch was decidedly more prominent than the uronic acid constituents extracted with it; and the accompanying quantities of pentoses were rather insignificant. In the hemicellulose extract, on the other hand, the lead in proportion of extractives rotated among the constituents in question.—HENRY OTTERSON AND W. E. TOTTINGHAM, *Department of Agricultural Chemistry, University of Wisconsin.*

DETECTION AND ESTIMATION OF FORMALDEHYDE WITHIN THE CELL OF A GREEN PLANT BY THE ALLISON APPARATUS

(WITH ONE FIGURE)

Most theories consider formaldehyde the first or at least an early step in the formation of sugars from carbon dioxide and water by the green

[3] Only one sample approached this departure, the next highest recovery being 114 per cent.

plant. The demonstration and estimation of this compound within the plant cell is therefore of great importance.

The inadequacy of the usual chemical methods is well presented by SPOEHR[1] and until now no satisfactory method has been found. The work of KLEIN and WERNER[2] and of POLLACCI and BERGAMASCHI[3] with dimedon appeared to give proof of the presence of formaldehyde in photosynthesizing plant cells. BARTON-WRIGHT and PRATT[4] have shown, however, that formaldomedon is formed when a solution of sodium bicarbonate is exposed to light in the absence of green plants.

Important factors that must be considered in a test to detect formaldehyde in photosynthesizing plant cells are: 1. Is the test specific? 2. Is it sufficiently sensitive? 3. Is the substance detected present in the normal cell and not, as may happen in macerated tissue, a decomposition product?

The ALLISON[5, 6] apparatus furnishes such a test. It gives readings specific for each compound in solution regardless of others that may be present. Compounds are detected without change. The requirements are that the solution must be sufficiently clear and uncolored to transmit adequate light to make the necessary observations, and that the concentration of the substance being detected is greater than three parts in 10^{12}. Detailed descriptions of the method[7] may be found in the literature, so that only enough will be given here to explain the present experiment.

The solution to be investigated is placed in a glass tube through which light passes, and if minima are obtained at scale readings characteristic of a specific compound, this compound is known to be present. If solutions are of sufficient dilution, quantitative determinations may be made with the aid of a circle so adjusted as to give the number of degrees through which the Nicol prism of the apparatus must be turned just to allow or just to prevent the appearance of the desired minima.

Since considerable light can pass through small unicellular algae, such as *Chlorella*, this type of organism appeared to be a means whereby the

[1] SPOEHR, H. A. Photosynthesis. pp. 289–291. Chemical Catalog Co. New York. 1926.

[2] KLEIN, G., and WERNER, O. Formaldehyde as an intermediate product in carbon dioxide assimilation. Biochem. Zeitschr. 168: 361–386. 1926.

[3] POLLACCI, G., and BERGAMASCHI, M. Demonstration with dimethylhydroresorcinol of formaldehyde in living plants during chlorophyll photosynthesis. Atti Accad. Lincei 10: 687–689. 1929.

[4] BARTON-WRIGHT, E. C., and PRATT, M. C. Studies in photosynthesis. I. The formaldehyde hypothesis. Biochem. Jour. 24: 1210–1216. 1930.

[5] ALLISON, FRED. Magneto-optic method of analysis as a new research tool. Ind. & Eng. Chem. Analytical Ed. 4: 9–12. 1932.

[6] ———————, and MURPHY, EDGAR. A magneto-optic method of analysis. Jour. Amer. Chem. Soc. 52: 3796–3806. 1930.

[7] *Loc. cit.*

presence of formaldehyde could be detected within the plant cell. The low light requirement of such organisms for photosynthesis also makes them suitable, since the amount of light coming through the apparatus is so small that it is doubtful whether it would be sufficient for most plants to begin or to continue this process.

In testing the algal[8] culture for formaldehyde, a tube containing distilled water was first inserted and observations made to determine the presence of formaldehyde. The test gave negative results. A drop of the algal suspension was then introduced and the observations repeated. Minima previously determined to be those of formaldehyde appeared (scale readings 21.83 and 21.92). The contents of the tube were then filtered through hardened filter paper into another tube and the observations repeated. The minima characteristic of formaldehyde were absent, showing that the formaldehyde present when the previous readings were made was within the algal cell. To preclude the possibility that the formaldehyde was adsorbed by the filter paper from the external solution and therefore only appeared to have been in the cells, a formaldehyde solution of about the same concentration as that found in the tube with the algae was made. This solution was filtered through the same type of filter paper and was found to contain the same amount of formaldehyde before and after filtration.

A brief study was made of the rate of formaldehyde formation as affected by the time of exposure to light. The algal cells to be studied were kept in the dark over night and until placed in the apparatus for observations the next morning. Since a small amount of light must pass through the substance examined, and the amount of light necessary for photosynthesis for such algae is small, an initial negative reading could hardly be expected. The first circle reading for the appearance of formaldehyde was 3.5°, corresponding to a concentration, considering the contents of the tube as a whole, of about 3.6 parts in 10^{12}.[9] This is about as small an amount as can be detected by this method. The tube and contents were then placed 2 feet from a 60-watt Mazda lamp for four periods of 7 minutes each and a final exposure of 15 minutes. Determinations were made after each exposure to determine the amount of formaldehyde present. The tube and contents were again placed in the dark and observations made the following morning. Ten-minute intervals of exposure were made. The first exposure was made 1 foot from a 25-watt Mazda lamp. The other exposures were made 2 feet from the same lamp as was used the preceding day. The results are shown in figure 1. Circle readings (increased readings show an

[8] Probably a species of *Chlorella*.

[9] This assumes that the angle corresponds to the same concentration for formaldehyde as for calcium. Details for quantitative determinations with the circle will be published later.

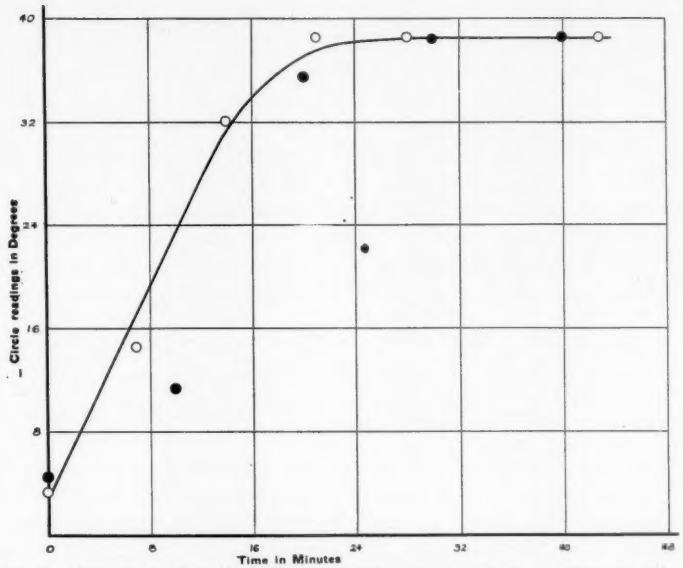

FIG. 1. Change in formaldehyde concentration with time on exposure to light: open circles, readings first day; solid circles, readings second day.

increase in concentration) were plotted against time of exposure. The final circle readings, 38.5°, correspond to a concentration of about 5 parts in 10^{10}. The algae were filtered out after the final observation and no formaldehyde was then found in the solution. The concentration within the algal cell could be determined only if the ratio of the amount of algae to the water in the tube were known and providing the effect were the same as if the formaldehyde were in the solution surrounding the algae. The fact remains, however, that up to a certain time the amount of formaldehyde in the cell increases on exposure to light of a certain intensity and after that remains the same, at least for the period observed.

This preliminary work suggests great possibilities, not only in further photosynthetic studies, but in determining various metabolic products of small organisms sufficiently transparent to allow enough light to pass through for work with the ALLISON apparatus. The minima for comparatively few organic compounds have been determined and the task of finding them may be long and tedious. Once these minima are found, the presence of compounds in solution in organisms of suitable types, such as bacteria and small, comparatively transparent fungi and algae, can easily be detected.—ANNA L. SOMMER, EDNA R. BISHOP, AND IRENE G. OTTO, *Alabama Agricultural Experiment Station, Auburn, Alabama.*

NOTES

Tenth Annual Meeting.—The tenth annual meeting of the American Society of Plant Physiologists will be held in Boston, Massachusetts, on December 28–30, 1933. The hotel headquarters is the Hotel Statler, Boston. The banquet will be held on Thursday evening at the Brunswick Hotel, Cambridge. A business meeting has been tentatively arranged for the afternoon of December 29. A large attendance is desirable at the business meeting, since it is constitutionally possible to transact business with final decisions, if the attendance is sufficiently large, without resorting to balloting by mail. As the banquet is the main social function of the meeting, members will find it desirable to arrive as early as possible, and to procure banquet tickets immediately on the opening day.

Program Committee.—The program committee for the Boston meeting consists of the following members: Dr. CARL G. DEUBER, Yale University; Dr. H. F. BERGMAN, United States Department of Agriculture; Dr. T. G. PHILLIPS, University of New Hampshire; Dr. DOROTHY DAY, Smith College; and Dr. B. E. GILBERT, Rhode Island Agricultural Experiment Station, chairman of the committee. The secretary is *ex officio* a member of the committee. Plans have been tentatively arranged for an attractive meeting which no one would wish to miss.

Membership Committee.—The membership committee, under the leadership of Dr. LAURENZ GREENE, of Purdue University, has been conducting an active campaign to hold and increase the membership of the Society. Other members of the committee are Dr. F. P. CULLINAN, U. S. Department of Agriculture; Dr. A. R. DAVIS, University of California; Dr. G. T. NIGHTINGALE, New Jersey Agricultural Experiment Station; and Dr. C. F. KORSTIAN, Duke University. The cooperation of all members in this work is highly desirable. If two new members were enlisted in each of the 48 states, the membership would reach a new high record. Special efforts to maintain and increase library support is desirable, also, since some of the more meagerly supported libraries found it necessary to discontinue subscriptions temporarily. Any member whose file is not complete as to available volumes can assist by purchasing back numbers as far as they are still obtainable. The first two volumes of PLANT PHYSIOLOGY have been exhausted for several years, and volumes three and four are scarce. The last four volumes are held in sufficient number to supply any probable demand for them in the near future. In order to main-

tain an uncongested outlet for publication of research in plant physiology, we should have support sufficient to publish about 1000 pages annually instead of 600.

Barnes Life Membership Committee.—The Barnes Life Membership committee has been appointed by Dr. C. O. APPLEMAN, president of the Society, and the selection of the candidate for the award will be made under the liberalized by-laws which were adopted at the time of the annual election last June. The committee consists of five members, as follows: Dr. WALTER F. LOEHWING, University of Iowa; Dr. S. V. EATON, University of Chicago; Dr. CHARLES E. SANDO, U. S. Department of Agriculture; Dr. GEORGE P. BURNS, University of Vermont; and Dr. T. G. PHILLIPS, University of New Hampshire, chairman. The selection is usually announced at the annual banquet, an event which adds much pleasure to that function.

Stephen Hales Address.—The third STEPHEN HALES address will be presented before the Society at the Boston Meeting by Dr. HUBERT BRADFORD VICKERY, to whom was awarded for his excellent work on vegetable proteins the third STEPHEN HALES prize at the ninth annual meeting at Atlantic City in 1932.

Early Collection of Dues.—Since it is necessary to use our funds for publication conservatively and with caution, the editors of PLANT PHYSIOLOGY will appreciate an early response to the secretary's notices of dues for 1934. The constitution makes October 1 the date at which the dues for the following calendar year become collectible. This provision was made to enable the secretary to make up a relatively complete subscription list by January 1, so that no loss might be incurred by sending the January number of the journal to those who find it necessary to discontinue membership. Early payment of dues gives the editors opportunity to plan the scale of publication for the succeeding calendar year with intelligence and with confidence in the continuity of support.

Jethro Tull.—This year, 1933, is the 200th anniversary of the publication of JETHRO TULL'S great book, Horse-Hoeing Husbandry, which was published in Dublin by A. RHAMES in 1733. The memorial committee of the Society has decided to include a brief memorial program at the tenth annual meeting in honor of this event.

Wound Compensation, Transplantation, and Chimaeras in Plants.—The press of Julius Springer, Berlin, has published an extensive mono-

graph by N. P. KRENKE entitled Wundkompensation Transplantation und Chimären bei Pflanzen, in a German edition, with N. BUSCH and O. MORITZ, of Kiel, as translator and editor respectively. It is no. 29 of the Monographien aus der Gesamtgebiet der Physiologie der Pflanzen und der Tiere. The first section of the volume deals with natural mechanical factors, and the second part with artificial (chirurgical) factors in the production of abnormal growths, transplantation, and chimaeras. The first section is relatively brief (pp. 1–138), the second part extensive (pp. 139–877). This second section considers such topics as the reaction of cells and tissues to wounding; wound compensation; the results of transplantations; and the phenomena of growth chimaeras. The final section and appendix touch upon the problems of immunity, internal therapy, and serological relations of grafted tissues to one another. A great deal of information has been brought together in this interesting field. It is a valuable source book for investigators of such problems. The prices at which the publisher quotes it are RM 88 in brochure, and RM 89.8 in cloth binding. Orders may be placed directly with the publishers.

Handbook of Plant Analysis.—The fourth and final volume of this extensive handbook by G. KLEIN has been issued in two volumes paged consecutively. The two volumes together have 1868 pages. There is a table of constants for known plant constituents (pp. 1441–1706), which gives the melting point, boiling point, solubility, and optical rotations as far as they are known.

The first half of volume IV takes up methods for determining amino acids, amides, amines, proteins, purine and pyrimidine compounds, nuclein bodies, alkaloids, cerebrosides, and some unclassified constituents. The second half takes up methods of enzyme determination, methods of studying fermentation (carbohydrases, esterases, proteases, nucleases, amidases, and desmolases), antigens and antibodies, plant hormones, and vitamines. Some special methods of biological analysis are included, such as water examination, soil analysis, fermentation media, nitrogen fractionation, fractionation and preparation of pure substances by chromatographic adsorption.

The four volumes of this work bring together the methods for all kinds of chemical investigations of plant materials. Those who attempt to use the work should not forget that in compiling these methods it has not been possible to consider them very critically. The field covered has been too broad for any one individual or even for the entire group of authors involved to do more than state the methods, without critical comments as to the degree of accuracy and as to the precautions which must be taken

in their use. Such critical studies will devolve upon the users of the work.

The publisher is Julius Springer, whose Vienna plant has been issuing this set. The prices quoted for the two halves of vol. IV are RM 190 for brochure, and RM 198 for cloth bound copies. Address Julius Springer, Schottengasse 4, Wien I, Austria.

Errata.—Members and subscribers are requested to note errata for volumes 7 and 8 at the close of the table of contents in this number.

GENERAL INDEX[1]

A

Absorption of nutrient salts by prune trees grown in solution cultures, 1
Acidity and alkalinity produced by changes in nitrogen, sulphur, and carbon cycles, 509
Adsorption, dye, test to show relation of hydrophilic colloids to hardiness, 275
Aeration and soil type, effect upon root systems of aquatic plants, 203
Alcoholic extracts of plant tissues, nitrogen changes in stored, 166
Alfalfa, hardiness in relation to hydrophilic colloids as shown by dye adsorption test, 275
  yield, influence of leaf destruction by sulphur dioxide and by clipping, 223
Alkalinity produced by changes in nitrogen, sulphur, and carbon cycles, 509
Ammoniacal nitrogen, influence upon intake of calcium by tobacco plants, 340
Analysis, handbook of plant, G. KLEIN, 486, 571
Anatomy, phloem, 82
  physiological, of citrus fruits, and quality of prepared juices, 35
Annual election, 485
  meeting, 173
Apparatus, ALLISON, 564
  bioelectric potentials, 264
  culture solution control, 553
  determination of chlorophyll, 321
  fumigation, 225
  oxidase measurements, 328
  suction tension, 186
  temperature control, 545
  tropograph, 337
Apple trees, growth of, and nutrient elements, 537
  wood, specific heat and percentage of water, 168
  wood, starch and hemicellulose extracts from, 561
Apples, changes in waxlike coating caused by spray treatment, 475
Aquatic plants, effect of soil type and aeration upon root systems, 203
Argentia chlorophyll of maize, 105
Asparagin, 145

B

Bacteria, root nodule, 345
BAILEY, L. H., How plants get their names, 488
BALDWIN, I. L., Root nodule bacteria, 345
BARNES award, 173
  Life Membership committee, 570
Bioelectric potentials of *Elodea* leaves, effect of light, 263
BISHOP, EDNA R., Detection and estimation of formaldehyde within cell of green plant by ALLISON apparatus, 564
BLUM, H. F., Photodynamically induced tropisms in plant roots, 525
Boron essential for growth of lettuce, 305
Bouteloua gracilis, effects of clipping, 428
BRAUNER, L., Das kleine pflanzenphysiologische Praktikum, 345
Bromus inermis, effects of clipping, 429
Brussels sprouts, relation of hydrophilic colloids to hardiness in, 275
BURKE, EDMUND, Nutrient elements used by leaves and growth of apple trees, 537
BURNS, G. R., Photosynthesis in various portions of spectrum, 247

C

Cabbage, hardiness in relation to hydrophilic colloids as shown by dye adsorption test, 275
Calcium determination, 470
  determination, indirect colorimetric method, 469
  intake by tobacco plants, 340

[1] Papers published in PLANT PHYSIOLOGY are indexed in *The Agricultural Index*, The H. W. Wilson Co., New York City.

CALFEE, R. K., Boron essential for growth of lettuce, 305
CAMP, A. F., Physiological anatomy, type, variety, and maturity of citrus fruits as affecting quality of prepared juices, 35
Carbon assimilation and chloroplastid pigments, 114
cycle, acidity and alkalinity produced by changes in, 509
Cell of green plant, detection and estimation of formaldehyde within, 564
CHASE, W. W., Biographical sketch of HENRI LOUIS DUHAMEL DUMONCEAU, 163
Chemical methods committee, 344
Chimaeras in plants, N. P. KRENKE, 570
Chlorophyll and iron in green and chlorotic pear leaves, 449
argentia, of maize, 105
method for quantitative determination, 321
Chlorotic pear leaves, quantitative relation between chlorophyll and iron, 449
Chlorovaporization, 190
Citrus fruits, physiological anatomy, type, variety, and maturity as affecting quality of prepared juices, 35
CLARK, N. A., Manganese and growth of *Lemna*, 157
Clipping, effect on development of grass seedlings, 425
influence of leaf destruction by, on yield of alfalfa, 223
Clover, influence of nitrogenous compounds and mannitol on nodule formation, 141
COLBY, H. L., Seasonal absorption of nutrient salts by prune trees grown in solution cultures, 1
Starvation and distribution of mineral nutrients in prune trees grown in culture solutions, 357
Colloids, hydrophilic, relation to hardiness in cabbage, as shown by dye adsorption test, 275
Colorimetric determination of potassium in plant tissues, 315
method for calcium determination, 469
Committee, executive, 174
finance, 174
membership, 485, 569
memorial, 174
on chemical methods, 344
on physical methols, 174
program, 569
Compensation, wound, in plants, N. P. KRENKE, 570
Concentrations of inorganic ions as related to growth of excised root-tips of wheat seedlings, 489
CONRAD, J. P., Acidity and alkalinity produced by changes in nitrogen, sulphur, and carbon cycles, 509
Corn, growth of x-rayed, 291
Correction of previous paper by STEWARD and PRIESTLEY: Movement of organic materials in plants, 482
Cotton propagation by means of grafts, 171
CRAFTS, A. S., Sieve-tube structure and translocation in potato, 81
CRUESS, W. V., Gasometric method of estimating oxidase activity, 327
Cultures, new method of arranging experiments with vegetables in sand, with flowing solution, 553
Cytisus, flowering date, 407

D

Dark reaction, 529
Date of flowering as affected by climatic temperature, 395
DEAN, B. ELIZABETH, Effect of soil type and aeration upon root systems of aquatic plants, 203
Defoliation, and hardiness of winter wheat, 297
Destruction, leaf, by sulphur dioxide and by clipping, influence on yield of alfalfa, 223
Detection of formaldehyde within cell of green plant by ALLISON apparatus, 564
Determination, colorimetric, of potassium in plant tissues, 315
of calcium, indirect colorimetric method, 469
of chlorophyll, method for quantitative, 321

Development of clipped grass seedlings, 425
DEXTER, S. T., Decreasing hardiness of winter wheat in relation to photosynthesis, defoliation, and winter injury, 297
Environmental factors and hardening of plants, 123
Disease of soy bean, leaf-wrinkle, 333
Distribution of mineral nutrients in prune trees grown in culture solutions, 357
Dues, early collection of, 570
DUHAMEL, HENRI LOUIS DUMONCEAU, biographical sketch, 163
Dulcitol, occurrence in *Irideae lamina-rioides*, 480
DUNN, S., Relation of hydrophilic colloids to hardiness in cabbage, brussels sprouts, and alfalfa plants as shown by dye adsorption test, 275
Dye adsorption test to show relation of hydrophilic colloids to hardiness, 275

E

Effect of light on bioelectric potentials of isolated *Elodea* leaves, 263
of soil type and aeration upon root systems of aquatic plants, 203
Election, annual, 485
Electric polarity of *Elodea* leaf, 265
Elements, nutrient, used by leaves, and growth of apple trees, 537
Elodea leaves, effect of light on bioelectric potentials, 263
EMMERT, E. M., Indirect colorimetric method for calcium determination, 469
Endosperm removal in green and argentia seedlings, 115
Environmental factors affecting argentia chlorophyll pattern, 106
factors and hardening of plants, 123
Errata, 344, 572
Estimation of formaldehyde within cell of green plant by ALLISON apparatus, 564
Excised root-tips of wheat seedlings, growth of, as related to concentrations of inorganic ions, 489
Executive committee, 174
Extracts, starch and hemicellulose, from apple wood, 561
EYSTER, W. P., Plastid studies in genetic types of maize: argentia chlorophyll, 105

F

FEHÉR, D., Untersuchungen über die Mikrobiologie des Waldbodens, 487
Finance committee, 174
FISHER, R. A., Statistical methods for research workers, 176
Flowering and length of day, 348
Formaldehyde, detection and estimation within cell of a green plant, 564
FRED, E. B., Influence of nitrogenous compounds and mannitol on nodule formation by clover, 141
Root nodule bacteria, 345
Fruit trees, hemicellulose in, 559

G

GADDUM, L. W., Physiological anatomy, type, variety, and maturity of citrus fruits as affecting quality of prepared juices, 35
GARNER, W. W., Responses of plants to relative length of day and night, 347
Gas injury to pure cultures of *Spirodela*, 479
Gasometric method of estimating oxidase activity, 327
Genetic types of maize: argentia chlorophyll, 105
GERDEL, R. W., Rapid colorimetric determination of potassium in plant tissues, 315
GIRTON, R. E., Plant physiology laboratory at Purdue University, 344
GLASS, H. B., Effect of light on bioelectric potentials of isolated *Elodea* leaves, 263
Grafts and propagation of cotton, 171
Grass seedlings, clipping and development, 425
Growth behavior of prune trees and nutrient absorption, 15
of apple trees and nutrient elements, 537
of excised root-tips of wheat seedlings

as related to concentrations of inorganic ions, 489
of *Lemna*, and manganese, 157
of lettuce, boron essential for, 305
stimulative effects of x-rays on plant, 287
Guttation experiments, 180

H

HALES, STEPHEN, address, 570
Handbook of plant analysis, G. KLEIN, 486, 571
Hardening of plants and environmental factors, 123
Hardiness in cabbage, relation of hydrophilic colloids as shown by dye adsorption test, 275
of winter wheat in relation to photosynthesis, defoliation, and winter injury, 297
HASSID, W. Z., Dulcitol in *Irideae laminarioides*, 480
HAWLITSCHKA, EVA, *Tribonema*, 346
Heat and percentage of water in apple wood, 168
Hemicellulose extracts from apple wood, 561
in certain fruit trees, 559
HILL, G. R., Influence of leaf destruction by sulphur dioxide and by clipping on yield of alfalfa, 223
HIMMEL, W. J., Tropograph and its use, 337
Holcus sorghum sudanensis, effects of clipping, 432
HOPKINS, E. W., Influence of nitrogenous compounds and mannitol on nodule formation by clover, 141
Leaf-wrinkle, a nutritional disease of soy bean, 333
Hydraulics, sap, 177
Hydrophilic colloids, relation to hardiness in cabbage as shown by dye adsorption test, 275

I

Indirect colorimetric method for calcium determination, 469
Influence of leaf destruction by sulphur dioxide and by clipping on yield of alfalfa, 223
of nitrogenous compounds and mannitol on nodule formation by clover, 141
Injury, gas, to pure cultures of *Spirodela*, 479
winter wheat, and hardiness, 297
Intake of calcium by tobacco plants, 340
International critical tables, 486
Ions, concentrations of inorganic, as related to growth of excised root-tips of wheat seedlings, 489
Irideae laminarioides, occurrence of dulcitol, 480
Iron in green and chlorotic pear leaves, 449

J

JACOBSON, H. G. M., Relative influence of nitrate and ammoniacal nitrogen upon intake of calcium by tobacco plants, 340
JEFFREY, R. N., Gasometric method of estimating oxidase activity, 327
Juices, physiological anatomy and maturity of citrus fruits as affecting quality of prepared, 35

K

KLEIN, G., Handbook of plant analysis, 571; Handbuch der Pflanzenanalyse, 486
Koeleria cristata, effects of clipping, 435
KRENKE, N. P., Wound compensation, transplantation, and chimaeras in plants, 570

L

Laboratory of plant physiology at Purdue University, 344
Leaf-wrinkle, a nutritional disease of soy bean, 333
Leaves, chlorotic pear, quantitative relation between chlorophyll and iron, 449
effect of light on bioelectric potentials, 263
nutritional elements used by, and growth of apple trees, 537

Lemna, growth of, and manganese, 157
Length of day and night, responses of plants to relative, 347
Lettuce, boron essential for growth, 305
Light, effect on bioelectric potentials of *Elodea* leaves, 263
periods, abnormal, 354

M

MACLAGAN, J. F. A., Date of flowering as affected by climatic temperature, 395
Maize, plastid studies in genetic types, 105
Manganese and growth of *Lemna*, 157
Mannitol, influence on nodule formation by clover, 141
Manual of plant biochemistry, W. E. TOTTINGHAM, 487
Manuscripts, 175
MARKLEY, K. S., Changes in waxlike coating of apples caused by spray treatment, 475
Maturity of citrus fruits as affecting quality of prepared juices, 35
McCOY, ELIZABETH, Root nodule bacteria, 345
McHARGUE, J. S., Boron essential for growth of lettuce, 305
Meeting, annual, 173
Atlantic City, 343
Boston, 485
Summer, 343, 485
Tenth annual, 569
Membership committee, 569
Memorial committee, 174
Method for quantitative determination of chlorophyll, 321
gasometric, of estimating oxidase activity, 327
indirect colorimetric, for calcium determination, 469
Methods, statistical, R. A. FISHER, 176
Microbiology of forest soils, D. FEHÉR, 487
Mineral nutrients, distribution in prune trees grown in culture solutions, 357
Minnesota section, 175
MITCHELL, J. W., Stimulative effects of x-rays on plant growth, 287
MORRIS, H. E., Nutrient elements used by leaves, and growth of apple trees, 537
MORRIS, V. H., Rapid colorimetric determination of potassium in plant tissues, 315
Movement of organic materials in plants: correction of previous paper by STEWARD and PRIESTLEY, 482

N

NEHRLING, H., Plant world in Florida, 488
Nitrate, relative influence upon intake of calcium by tobacco plants, 340
Nitrogen changes in stored alcoholic extracts of plant tissues, 166
compounds and mannitol, influence on nodule formation by clover, 141
cycle, acidity and alkalinity produced by changes in, 509
Nodule formation by clover, influence of nitrogenous compounds and mannitol, 141
Nutrient elements used by leaves and growth of apple trees, 537
salts, seasonal absorption by prune trees grown in solution cultures, 1
Nutrients, mineral, distribution in prune trees grown in culture solution, 357
Nutritional disease of soy bean, leaf-wrinkle, 333

O

Oats, growth of x-rayed, 294
OLTMAN, R. E., Method for quantitative determination of chlorophyll, 321
OSERKOWSKY, J., Quantitative relation between chlorophyll and iron in green and chlorotic pear leaves, 449
OTTERSON, HENRY, Examination of starch and hemicellulose extracts from apple wood, 561
OTTO, IRENE G., Detection and estimation of formaldehyde within cell of green plant by ALLISON apparatus, 564
Oxidase activity, gasometric method of estimating, 327
Oxygen requirement and tropisms, 534

P

Pear leaves, chlorotic, quantitative relation between chlorophyll and iron, 449
Percentage of water in apple wood, and heat, 168
Phloem anatomy, potato, 82
Phosphate determination, 470
Photodynamics and plant roots, 525
Photoperiodism, 347
Photosynthesis, and hardiness of winter wheat, 297
in various portions of spectrum, 247
Phototropism in plant roots, 525
Physical methods committee, 174
Physiological anatomy, type, variety, and maturity of citrus fruits as affecting quality of prepared juices, 35
Pigments, chloroplastid, in argentia plants, 111
Plant, detection and estimation of formaldehyde within cell of a green, 564
growth, stimulative effects of x-rays, 287
names, L. H. BAILEY, 488
tissues, colorimetric determination of potassium, 315
tissues, nitrogen changes in stored alcoholic extracts, 166
world in Florida, H. NEHRLING, 488
Plants, responses to relative length of day and night, 347
wound compensation, transplantation, and chimaeras in, 570
Plastid studies in genetic types of maize: argentia chlorophyll, 105
Poa pratensis, effects of clipping, 439
Polarity, electric, of *Elodea* leaf, 265
Portraits, DUHAMEL DUMONCEAU, opp. p. 163
Potassium in plant tissues, colorimetric determination, 315
Potato, sieve-tube structures and translocation, 81
Potentials, bioelectric, of isolated *Elodea* leaves, 263
PRIESTLEY, J. H., Movement of organic materials in plants: correction, 482
Principles of plant physiology, O. L. RABER, 487
Prize, STEPHEN HALES, 343
Program, committee, 569
Propagation of cotton by means of grafts, 171
Prune trees grown in culture solution, distribution of mineral nutrients, 357
trees grown in solution cultures, seasonal absorption of nutrient salts by, 1
Publication, support of, 344
Purdue University Plant Physiology Laboratory, 344
section, 486

Q

Quality of prepared juices of citrus fruits, 35
Quantitative determination of chlorophyll, 321
relation between chlorophyll and iron in green and chlorotic pear leaves, 449
Quantum yields, in photosynthesis, 260

R

RABER, O. L., Principles of plant physiology, 487
REA, H. E., Field propagation of cotton by means of grafts, 171
Responses of plants to relative length of day and night, 347
Rhododendron, flowering date, 401
ROBERTSON, J. H., Effect of clipping on development of grass seedlings, 425
Root nodule bacteria, 345
systems of aquatic plants, effect of soil type and aeration, 203
-tips, excised, or wheat seedlings and concentrations of inorganic ions, 489
Roots and photodynamically induced tropisms, 525

S

SAEGER, A., Gas injury to pure cultures of *Spirodela*, 479
Salts, nutrient, seasonal absorption by prune trees grown in solution cultures, 1

Sand cultures with flowing solution, new method of arranging experiments with vegetables in, 553
SANDO, C. E., Changes in waxlike coating of apples caused by spray treatment, 475
Sap hydraulics, 177
SCOTT, K. G., Photodynamically induced tropisms in plant roots, 525
Seasonal absorption of nutrient salts by prune trees grown in solution cultures, 1
Section, Minnesota, 175
Purdue, 486
Seedlings, wheat, growth of excised root-tips and concentrations of inorganic ions, 489
SHULL, C. A., Stimulative effects of x-rays on plant growth, 287
Sieve-tube structure and translocation in potato, 81
Soil type and aeration, effect upon root systems of aquatic plants, 203
Soils, microbiology of forest, D. FEHÉR, 487
Solution cultures, seasonal absorption of nutrient salts by prune trees, 1
new method of arranging experiments with vegetables in sand cultures with flowing, 553
SOMMER, ANNA L., Detection and estimation of formaldehyde within cells of green plants by ALLISON apparatus, 564
Soy bean, nutritional disease, 333
Spectrum, photosynthesis in various portions, 247
Spirodela, gas injury to pure cultures, 479
Spray treatment and changes in waxlike coating of apples, 475
STAHL, A. L., Physiological anatomy, type, variety, and maturity of citrus fruits as affecting quality of prepared juices, 35
Starch extracts from apple wood, 561
STARK, A. L., Correlation of specific heat and percentage of water in apple wood, 168
Starvation and distribution of mineral nutrients in prune trees grown in culture solutions, 357
Statistical methods, R. A. FISHER, 176
STEPHEN HALES prize, 343
STEWARD, F. C., Controlled thermostats for physiological studies at low, non-freezing temperatures, 545
Movement of organic materials in plants: correction, 482
Stimulative effects of x-rays on plant growth, 287
Stipa spartea, effects of clipping, 440
Stored alcoholic extracts of plant tissues, nitrogen changes, 166
Structure, sieve-tube, and translocation in potato, 81
Sulphur cycle, acidity and alkalinity produced by changes in, 509
dioxide, leaf destruction by, and yield of alfalfa, 223
Summer meeting, 485
Sunflower, growth of x-rayed, 294
Support, 174
SWANBACK, T. R., Relative influence of nitrate and ammoniacal nitrogen upon intake of calcium by tobacco plants, 340
Syringa, flowering date, 415

T

Temperature, climatic, and date of flowering, 395
interrelation of length of day, 353
Temperatures, controlled thermostats for physiological studies at low, non-freezing, 545
Tensile column theory, 185
Thermostats, controlled, for physiological studies at low, non-freezing temperatures, 545
THOMAS, M. D., Influence of leaf destruction by sulphur dioxide and by clipping on yield of alfalfa, 223
Tissues, plant, colorimetric determination of potassium, 315
plant, nitrogen changes in stored alcoholic extracts, 166
Tobacco plants, and intake of calcium, 340
TOTTINGHAM, W. E., Character of hemicellulose in certain fruit trees, 559

Examination of starch and hemicellulose extracts from apple wood, 561
Manual of plant biochemistry, 487
Translocation, 482
in potato, and sieve-tube structure, 81
Transplantation in plants, N. P. KRENKE, 570
TRAUB, H. P., Physiological anatomy, type, variety, and maturity of citrus fruits affecting quality of prepared juices, 35
Trees, hemicellulose in certain fruit, 559
Tribonema, 346
Tropisms, photodynamically induced in plant roots, 525
Tropograph and its use, 337
TULL, JETHRO, 570

U

Urea, 145

V

Vegetables, new method of arranging experiments with, in sand cultures with flowing solutions, 553

W

Water in plant wood, percentage, and heat, 168
Waxlike coating of apples, changes caused by spray treatment, 475
WEBSTER, J. E., Nitrogen changes in stored alcoholic extracts of plant tissues, 166
WETTSTEIN, FRITZ VON, Fortschritte der Botanik, 345
Wheat, growth of x-rayed, 290
seedlings, concentrations of inorganic ions as related to growth of excised root-tips, 489
winter, decreasing hardiness in relation to photosynthesis, defoliation, and winter injury, 297
WHITE, P. R., Concentrations of inorganic ions as related to growth of excised root-tips of wheat seedlings, 489
Wood, apple, heat and percentage of water, 168
apple, starch and hemicellulose extracts from, 561
WOODHOUSE, E. D., Sap hydraulics, 177
Wound compensation in plants, 570

X

X-rays, stimulative effects on plant growth, 287

Y

Yield of alfalfa, influence of leaf destruction by sulphur dioxide and by clipping, 223

Z

ŻURBICKI, Z., New method of arranging experiments with vegetables in sand cultures with flowing solution, 553